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THE JOHNS HOPKINS HOSPITAL WITH ESPECIAL REFERENCE TO ITS RELATIONS TO MEDICAL EDUCATION AND THE PROMOTION OF MEDICAL KNOWLEDGE

by

WILLIAM H. WELCH

WITH AN EXPLANATORY NOTE

by

ALAN M. CHESNEY

(*Dean Emeritus of the Medical Faculty, The Johns Hopkins University*)

THE EXPLANATORY NOTE

The statement by Dr. William H. Welch which is reprinted herewith was submitted to Mr. Starr J. Murphy of New York City, legal counsel to Mr. John D. Rockefeller, Sr., in February, 1904, shortly after the Johns Hopkins Hospital had suffered a severe loss in its endowment through the destruction, in the Great Baltimore Fire of 1904, of many buildings which it owned and from the rental of which it derived a substantial part of its income.

The authorities of the Hospital had appealed to Mr. Rockefeller for aid and he had sent Mr. Murphy to Baltimore to determine for himself the extent of the impairment of the Hospital's finances. After his return to New York Mr. Murphy received an official statement from Judge Harlan, President of the Board of Trustees of the Hospital, setting forth the extent of the Hospital's losses which were calculated at approximately half a million dollars, taking into account both capital and income.

At the same time Dr. Welch, who was then Pathologist to the Hospital and Professor of Pathology in the School of Medicine, drew up the statement quoted below and submitted it to Mr. Murphy through Dr. Henry M. Hurd, then Superintendent of the Hospital. Dr. Welch requested Dr. Hurd to forward with the document "a statement of expenditures by the Hospital for salaries of those whose work is connected both with the Hospital and the Medical School".[1]

Dr. Welch's statement was duly forwarded by Dr. Hurd to Mr. Murphy and was subsequently quoted in part by Dr. Hurd in his annual report for the year ending January 31, 1904, but for some reason which is not at all clear to the writer, he did not give Dr. Welch credit for its authorship in that report. He merely stated that it had been "prepared by a member of the Hospital Staff . . .", although the original document was signed by Dr. Welch![2]

[1] Chesney, Alan M.: The Johns Hopkins Hospital and The Johns Hopkins University School of Medicine, Vol. II, page 382.

[2] Hurd, Henry M.: Fifteenth Report of the Superintendent of The Johns Hopkins Hospital. Baltimore, The Johns Hopkins Press, 1904, pp. 15–22.

As a result of this omission the identity of the author of the statement remained generally unknown and the statement itself did not therefore appear in the volumes of collected papers and addresses of Dr. Welch which appeared under the editorship of Dr. Walter C. Burket in 1920. Indeed, it was not until the spring of 1957, that a search through the collection of Welch papers which the late Dr. Simon Flexner deposited in the Welch Medical Library following the completion of the biography of Dr. Welch by himself and his son, Mr. James Thomas Flexner, revealed the fact that Dr. Welch was the author of the statement, that the original manuscript was in his own handwriting, and that it was in the possession of Mr. John D. Rockefeller, Jr. Overtures to Mr. Rockefeller led to his kind permission to publish the statement in full, and it has already appeared in the second volume of the official history of The Johns Hopkins Hospital and The Johns Hopkins University School of Medicine.

It has seemed desirable to reprint this contribution by Dr. Welch for two particular reasons.

In the first place any authentic manuscript by a person of Dr. Welch's distinction that has not been published in full, or that has been published in such a way as not to disclose his authorship, seems worthy of publication in its own right and deserves to be brought to general attention.

In the second place the statement presents so clearly, concisely, and fully, the attitude of the authorities of The Johns Hopkins Hospital of 1904 toward those two closely related activities, medical education and medical research, that it may be fairly said to constitute the intellectual charter of the institution. As such, it deserves study by all those who are interested in, or have a responsibility for, the conduct of The Johns Hopkins Hospital.

STATEMENT

"The relations of the Johns Hopkins Hospital to the Medical Department of the University and the resulting efforts for the improvement of medical education and the advancement of medical knowledge have been and will continue to be the most distinctive features of its work. These relations and aims have controlled the policy of the Trustees from the beginning, and that such was the intention of the founder of the institution is clearly indicated in his letter to them quoted in the Annual Catalogue and Announcement of the Medical Department (1903–1904), page 85. These purposes required that the Hospital should represent in its construction the best and most advanced ideas in all that pertains to sanitary arrangements and facilities for the care of the sick and injured, and in its organization and operation the most effective means for the clinical training of medical students and physicians, for the investigation of disease, and for the treatment of patients. Essential for the full attainment of these aims was association with a university with high educational ideals.

Feb 1904 Johns Hopkins Hosp.

The Johns Hopkins Hospital with especial reference to its Relations to Medical Education and the Promotion of Medical Knowledge.

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.....

We have every hope and confidence that the Medical School, the Hospital, and the University will be enabled to continue their useful work and to expand it in the future.

William H. Welch.

1. Construction of the Hospital

"The Hospital was designed with especial reference to its uses for higher medical education and research. It was intended to represent the best in hospital construction with the main emphasis placed upon the sanitary arrangements, such as heating, ventilation, facilities to secure thorough cleanliness and to prevent the spread of infection. That this intention was fulfilled in very large measure is indicated by the international reputation of the Hospital as a model of hygienic construction, by the influence which it has had upon the building of later hospitals, and by the conspicuous place which the plans of the Hospital occupy in the recent treatises devoted to the discussion of the methods and problems of hospital construction, such as in H. C. Burdett's great work on 'Hospitals and Asylums of the World', London, 1891–3, and F. Ruppel's 'Anlage und Bau der Krankenhäuser', Jena, 1896. Admirably located upon high and ample grounds and devoid of unnecessary architectural adornments the buildings of the Hospital are impressive in their simplicity, substantial construction and manifest adaptation to their needs.

2. Medical Organization of the Hospital

"The plan of organization of the medical staff of the Hospital differs in important respects from that which prevails in other general hospitals in this country, and this has been one of the main instruments which has enabled the Hospital and Medical School to accomplish what they have done for the furtherance of higher medical education and research. The intimate association of the Hospital with the Medical Department of the University secures on the one hand to the Hospital the professional services of the teachers in the Medical School, and on the other hand to the School advantages for clinical training of a kind not at present enjoyed in equal measure by any other medical school in this country.

"The Superintendent of the Hospital, who holds also the professorship of psychiatry in the University, is a medical man in thorough sympathy with the educational and scientific aims of the institution.

"At the head of each of the five principal divisions of the Hospital—medical, surgical, gynecological, obstetrical, and pathological—stand the professors in the University of the corresponding branches, who have entire professional control of their respective departments under the authority of the Medical Board (constituted by them and the Superintendent) and of the Trustees.

"Next to these chiefs of departments comes, as a distinctive feature, a staff of resident and assistant resident physicians, surgeons, gynecologists, obstetricians, and pathologists, numbering at present sixteen in all, and finally under these twelve resident house officers, this latter group corresponding to the internes of other hospitals.

"The establishment of the group of residents and assistant residents over the ordinary hospital internes offers especial advantages. The former positions are open to graduates of other medical schools, and have been held in many instances by such graduates, who have already completed as a rule an internship in some hospital. The appointments, while subject to annual renewal, may be held for an indefinite period. The young physicians who are so fortunate as to secure these positions are in general picked men attracted by the unusual opportunities for advanced clinical training. The opportunities open to young physicians in this country to fit themselves for the higher clinical positions are at present lamentably few, and one of the most important fields of usefulness of the Johns Hopkins Hospital has been in the direction of meeting this need, so far as its resources permitted. The results of a considerable part of the scientific work of the Hospital are to be found in the publications of this specially selected and highly trained group of young physicians, who by the time they are ready to leave their positions in the hospital have in many instances acquired an experience and reputation which enable them to enter at once upon careers of great promise.

3. Relation of the Hospital to Medical Education

"The opening of the Johns Hopkins Medical School in 1893 marked a universally recognized and urgently needed advancement in the standards and methods of medical education in this country. The requirements for admission established by the special endowment of the School and consisting in a liberal degree in arts or science, a specified practical training in the sciences fundamental to medicine (physics, chemistry, and biology) and a reading knowledge of French and German, are as high as, if not higher than those of any medical school in the world. Doubtless the time has not come for the general adoption in this country of equally high requirements, but there is room for a few schools with such standards. The increasing prosperity of the Medical Department of the Johns Hopkins University as regards the attendance of students has been due in no small measure to the severe requirements for admission, which have been an incentive rather than a bar to a superior grade of students.

"The reform in the methods of teaching introduced by the Medical School consisted mainly in the abandonment of didactic lectures as the principal mode of instruction and the adoption for every subject taught of courses of practical, personal training in the laboratories or in the Dispensary and the Hospital. The School is supplied with well equipped laboratories. There has been no more remarkable and gratifying feature in the progress of medical education in America during the last twelve or fifteen years than the rapid increase in the opportunities for satisfactory laboratory training, so that it can be said that, while the country needs more and better medical laboratories, six or eight of

our best medical schools are supplied with facilities for laboratory instruction which compare favorably with those in leading European universities.

"At present the weakness even in our better American medical schools is not so much on the laboratory side as it is in the lack of correspondingly good opportunities for the training of undergraduate medical students in hospitals during the last two years of their course. What is needed is much more than attendance upon clinics held in an amphitheatre or even upon classes permitted to accompany an instructor in the hospital wards. The student should be brought directly in contact with patients whom they are permitted to examine.

"It is at present the singular advantage of the Johns Hopkins Medical School among American schools that through its association with a large, well equipped and endowed general hospital it is enabled to supply the kind of clinical training which has just been indicated as essential to the thorough education of medical students. The need of such a relationship between a medical school and a large hospital is now so generally appreciated that it is to be expected and desired that other medical schools should secure similar advantages.

"The details of the methods of practical teaching in the Dispensary and Wards of the Hospital are set forth in the Annual Catalogue and Announcement (1903–4, pp. 53, 54, 65 et seq.).

"It is apparent from what has been said how essential to the Medical School is its relation to the Hospital and how seriously impaired would be its usefulness by any curtailment of the opportunities now offered by the Hospital for clinical training and utilized to the fullest extent.

"Mention has already been made of the special advantages afforded by the plan of organization of the Hospital to selected young physicians for advanced clinical research and work. It may be added that special courses for physicians are given during May and June of each year and are attended usually by from sixty to seventy practitioners who come from all parts of the country and especially from the South and West. Opportunities are also supplied to suitably trained graduates in medicine to undertake special and advanced work in the laboratories and the hospital.

"A glance at the geographical distribution and college graduation of the undergraduate students, as given in the Annual Catalogue (p. 37), will show how nationally representative in this respect is the Medical School.

"The Clinical Laboratory connected with the Hospital was established by a gift of $10,000 and has been of very great service. Through this and other laboratory facilities the application of those microscopical, chemical and bacteriological procedures which in recent years have acquired such great importance for the diagnosis and even for the treatment of disease, can be practiced and taught.

"Other useful gifts received by the Hospital and Medical School for educational and scientific purposes are the fund for the study of tuberculosis and

the endowment of the Herter Lectureship by Dr. and Mrs. Christian A. Herter (Annual Announcement, pp. 91 and 92).

4. Relation of the Hospital to the Advancement of Medical Knowledge

"The Trustees of the Hospital and the University as well as the members of the medical faculty and of the Hospital staff have recognized from the outset that one of the chief functions of a great hospital and medical school should be the improvement of medical knowledge. A controlling consideration in the selection of the professors in the Medical School has been the possession of ability to conduct and to stimulate investigations in the science and art of medicine. As a rule those with the capacity and the desire for original research are also the best teachers, especially when the methods of instruction are personal and practical, and an atmosphere of independent investigation and work has in itself a high educational value to the student.

"It is hardly possible nor does it seem necessary in this statement to enumerate the hundreds of papers and monographs embodying the results of work done in the various laboratories of the Medical School and in the Hospital, nor even to point out the particular value of these publications. The international reputation of the Johns Hopkins Hospital and Medical Department is based largely upon these contributions, and it is this side of the work of these institutions which is most widely known and appreciated. Many of the larger monographs will be found in the 'Reports of the Johns Hopkins Hospital' now in their twelfth volume, and the shorter papers in the 'Bulletin of the Johns Hopkins Hospital' now in the fifteenth volume, but much of the work has appeared also in various medical journals and in books. Contributions have been made both to the various medical sciences and to medical and surgical practice in its different branches. Among the contributions from the Hospital of especial practical importance may be specified those relating to amoebic dysentery, blastomycetic dermatitis, the malarial fevers, typhoid fever, disinfection of the skin and other matters of surgical technique, the radical treatment of cancer of the breast, the radical cure of inguinal hernia, methods of intestinal suture, cancer of the uterus, catheterization of the ureters, diabetes and diseases of the pancreas. It is of course to be understood that the foregoing is not intended as a complete summary.

"The Trustees have adopted a liberal policy in supplying means for original investigations and in supporting the publication of the 'Reports' and the 'Bulletin' and also in providing for drawings and illustrations. It is believed that in no direction have the expenditures yielded more valuable returns.

"It is undoubtedly the reputation of the teachers and the stimulus and opportunities for original research and advanced work which have attracted to the Medical School and the Hospital so many bright and well trained young physicians and students.

"There remain a few other considerations to which it may be pertinent to refer briefly, although this statement is intended mainly to point out the more important relations of the Hospital to education and research.

5. The Treatment of Patients

"The Johns Hopkins Hospital is primarily a great charitable foundation and the welfare of the patient is of course always the first consideration. It is hardly necessary to state that the educational and scientific features of the work of the Hospital conduce directly to improved methods of diagnosis and treatment. Being supported by private endowment the Hospital can select its patients. While this selection is determined mainly by the likelihood of benefitting the patient, cases of special interest or importance for investigation and study and for the instruction of students are admitted even when a fatal issue is probable. No attempt is made to select cases solely with reference to presenting at the end of the year a low death-rate.

"While resorted to by patients from all parts of the country the Hospital has been a special benefaction first in order to residents of Baltimore and Maryland, and next to those of the South and Middle West.

6. The Training School for Nurses

"The Hospital maintains an excellent training school for nurses with a three year's course preceded by a period of apprenticeship during which the fitness of the candidate can be determined. This School has been active in improving the standards and training methods for this important and useful field of professional work for women.

7. Coeducation

"By the terms of the special endowment of the Medical School women are admitted and enjoy all the advantages of the School upon the same terms as men. The proportion of women students has not been large and shows no tendency to increase. While at the beginning there were some doubts expressed as to the wisdom of the coeducational feature of the School, experience has shown that they were not justified. There is no probability that coeducation would be abandoned if the matter were now left to the free choice of the Medical Faculty. The presence of women students has elevated the general tone of the Medical School. A certain number of women physicians are needed by the community, and it is highly desirable that they should have opportunities for medical education equal to those supplied to men. There would be great lack of economy in making separate provision for the medical education of women in view of the large expense of such education. Two or three of our women graduates appear to be equal to the best of the men in ability and achievement. Women are eligible for the interne positions in the Hospital which is one of

the very few hospitals offering such opportunities for clinical training to their sex. The lack of these opportunities has been a main obstacle to the thorough professional training of women in medicine.

8. Relations of the Medical School to the University and to the Hospital

"The Medical School is upon the University foundation controlled by the Trustees of the University. It is in every sense an integral part of the University, coordinate with the other Faculties. The President of the University has been from the beginning a member of the Medical Faculty and has been active and most helpful in its councils. This association of the Medical Department with a university of the reputation and the ideals of the Johns Hopkins University has been of inestimable mutual benefit.

"Since the opening of the Medical School in 1893 the University has not been able to aid the School by expenditures from its own special funds. The School could not have been started without the support of a special endowment which was secured largely through the efforts of women and particularly through the generosity of Miss Mary Garrett (see Annual Catalogue and Announcement, 1903–4, pp. 86–89). Through this endowment, the important assistance supplied by the Hospital and the tuition fees the Medical School is now self-supporting.

"The salaries and other expenditures for the support of those departments, such as anatomy, physiology, physiological chemistry and pharmacology, not directly concerned with the work of the Hospital, are paid by the University out of the income derived from the endowment of its Medical Department and the tuition fees.

"The salaries and other expenses for the maintenance of the departments directly concerned with the work of the Hospital as well as with that of the Medical School, such as pathology, medicine, surgery, gynecology, obstetrics, are paid partly by the Hospital and partly by the Medical School. The distribution of these expenditures is given in an accompanying statement supplied by the Superintendent of the Hospital.

"It is evident that independently of the use of the Hospital by the Medical School for clinical teaching the financial aid received directly from the Hospital is of great importance to the Medical School and at present essential for its maintenance.

"It is believed that the principle underlying the foregoing distribution of expenditures between the Hospital and the University is the correct one. In a University Medical School the professors of the great practical branches of medicine should give their main attention to the work of teaching, of investigation and of hospital practice, and should not engage in an extensive private and family practice. None of these professors in the Johns Hopkins Medical School have other than outside consulting practice. Their principal professional

work is within the Hospital where they spend much more time than is the custom of attending physicians in most hospitals. For this it is proper that they should be paid by the hospital, and by such payment the Hospital is entitled to receive the best services in expenditure of time, energy, thought and devotion to its interests from the members of its medical staff. In this way the interests both of the Medical School and of the Hospital are best served.

"Although the University and the Hospital are under separate Boards of Trustees, there are several members in common upon both Boards. These Boards have always worked in entire harmony with each other. The relations of the Hospital to the Medical Department of the University are so clearly set forth in the letter of Johns Hopkins, are so thoroughly established in practice and are so definitely and unanimously agreed upon by the two Boards that there can be no apprehension that they will ever be disturbed.

9. Growth and Future Development of the Medical Department

"The Medical School was opened in 1893, with only the first year of the course fully organized, with an entering class of eighteen. With the completed organization of the full four years' course in 1896 the total number of students matriculated in the School was one hundred and twenty three. By that time it became apparent that the high standards of admission would not restrict unduly the attendance upon the school.

"At the date of publication of the last annual catalogue in April 1903 the total enrollment of students studying for the degree of Doctor of Medicine was two hundred and fifty seven (Annual Catalogue, p. 36), and this enrollment for the current academic year is two hundred and seventy five.

"This growth of the Medical Department, while most gratifying, makes at present demands upon the accommodations and facilities for teaching in the Pathological Laboratory and the Hospital, which reach almost the limits of their present capacity, so that any restriction whatever of these opportunities would be a very serious embarrassment to the Medical School. The suggestion has been made that without enlarged accommodations and facilities we may be called upon to limit in some way the number of students, but there is an earnest desire to avoid, if possible, such a course, nor is such a policy at present contemplated.

"We have every hope and confidence that the Medical School, the Hospital, and the University will be enabled to continue their useful work and to expand it in the future.

William H. Welch"

KARTAGENER'S TRIAD: A FAMILY STUDY[1]

GEORGE W. GORHAM AND JOHN G. MERSELIS, JR.[2]

Department of Medicine, The Johns Hopkins University School of Medicine and Hospital

INTRODUCTION

In 1933 Kartagener (14) described four patients with coexisting bronchiectasis, situs inversus, and sinusitis. Although similar cases had been reported by Siewert (25) in 1903 and by Gunther (10) in 1923, Kartagener was the first to recognize the association of these abnormalities.

Bergstrom (3) *et al.* in 1950, studied a family in which two of six siblings had Kartagener's Triad, two had bronchiectasis, and two were normal. The parents were in good health. Prior to this study there were three other recorded instances where more than one member of a family had this syndrome. Gunther described two sisters among four siblings with these abnormalities, Kartagener (13) reported the triad in two sisters, and Lopez (17) found three siblings with situs inversus, bronchiectasis, and sinusitis.

The purpose of this paper is to present two cases of Kartagener's Triad occurring among siblings with a detailed study of their family tree considering the simultaneous occurrence of bronchiectasis, sinusitis, and situs inversus. In addition, a review of 38 cases of Kartagener's Triad reported since 1950 is undertaken to determine the incidence and distribution of the triad among family members. The occurrence of the syndrome among 6 families, including 5 previously reported (7, 11, 12, 15, 24), is shown in Table I.

CASE REPORTS

Case 1.

O. B. (JHH 752602)—a 25 year old white male, was admitted to The Johns Hopkins Hospital on October 10, 1956, with a long history of productive cough, "asthmatic attacks", and moderate exertional dyspnea. For one month prior to admission he had noted exacerbation of the chronic cough, increasing dyspnea and wheezing, and purulent sputum. During this period there was also ready fatigability, generalized weakness, anorexia, and 20 pounds weight loss. The week before admission he developed pleuritic left chest pain and paroxysms of coughing which yielded 2 to 3 cups of foul, green sputum per day. There were several episodes of hemoptysis.

Physical examination on admission revealed: T 103° P 148 R 48 BP 95/70. The patient was a poorly-nourished and poorly-developed acutely ill man in severe respiratory distress coughing up copious amounts of foul sputum. He was extremely dehydrated. There was early clubbing of the fingers and toes, and evidence of marked pulmonary consolidation over

[1] The work was supported by a Training Grant (2E-9) with the National Institute of Allergy and Infectious Disease.

[2] Requests for reprints should be sent to Dr. John Merselis, The Johns Hopkins Hospital, Baltimore, Md.

TABLE I

Reports of the Familial Character of Kartagener's Triad

Author	Family Members Studied	Presence of Triad	Related Abnormalities	Kartagener's Triad Established by:		
				Hist.	P.E.	X-Ray
Torgersen	2 siblings	+		+	+	+
	"relatives"	0	0			+
Karani	2 siblings	+		+	+	+
	father	0	sinusitis	+		
	2 siblings	0	1 sibling with "left-sided" heart	+		
	offspring of pt. with Kartagener's	0	0	+		
Dickey	2 siblings; others not studied	+	+	+	+	+
Kaye	2 siblings	+		+	+	+
	parents	0	0			+
	2 siblings	0	0	+		
Katz	2 siblings; others not studied	+	+	+	+	+
Gorham and Merselis	2 siblings	+		+	+	+
	parents	0	0	+	+	+
	3 siblings	0	0	+	+	+
	1 paternal uncle	0	0	+	+	+
	1 nephew	0	0	+	+	+
	1 nephew	0	0	+	+	
	1 niece	0	0	+	+	
	4 paternal grandparents	0	0	+		
	4 maternal grandparents	0	0	+		
	6 maternal uncles	0	0	+		
	5 maternal aunts	0	0	+		
	2 paternal uncles	0	0	+		
	1 paternal aunt	0	0	+		

the lower left chest. Tubular breathing, coarse wheezes, and crepitant rales were noted over both lung fields. The cardiac impulse was palpable at the left sternal border, but the heart could not be outlined because of the pulmonary congestion. Auscultation of the heart revealed no murmurs, rubs, or abnormal rhythm. There was some left upper quadrant tenderness and guarding but no rebound. The remainder of the examination was unremarkable.

Admission laboratory data revealed a hematocrit (Wintrobe method) of 42.5 per cent, icteric index 7.5, erythrocyte sedimentation rate 31 mm./hour corrected to 23 mm./hour, and a WBC count of 30,050 with a marked shift to the left. The urine was normal. X-ray studies revealed extensive bilateral "honeycomb" infiltration involving both lower lung fields and obscuring the left cardiac border. The heart was noted to lie largely in the right hemithorax and the aortic arch was prominent on the right side. True dextrocardia was confirmed by electrocardiogram. Bronchiectasis was considered certain, but the patient refused a broncho-

graphic examination. Pansinusitis was subsequently demonstrated radiologically, and complete situs inversus was verified by barium swallow.

The clinical course was one of gradual defervescence over a seven day period accompanied by very slow resolution of the pneumonia. Coarse rales and wheezes persisted throughout hospitalization despite vigorous antibiotic therapy and postural drainage. The WBC count fell steadily to 12,000 and the CO_2 combining power ranged from 31.8 to 36.8 mEq./L. Only normal bacterial flora were grown from repeated sputum cultures. Radiologic examinations showed gradual clearing of the right chest, but the pneumonitis remained and bullae were noted on the left side. Bronchoscopy was felt to be therapeutically indicated but was refused repeatedly by the patient who left the hospital against advice on the 31st day.

Case 2.

M. W. (JHH 754176)—age 24 stated that she was "born with a cold" and gave a history of frequent respiratory infections since infancy. She had a tonsillectomy and adenoidectomy at age 6 without subsequent improvement and pneumonia at 17. During childhood she had numerous attacks of sinusitis accompanied by otitis media on several occasions. There was some secondary hearing loss. She complained of constant nasal discharge and a morning cough productive of sputum which was sometimes purulent. Aching chest pain had accompanied several of her more severe respiratory infections, and there had been one episode of hemoptysis. Exercise tolerance had always been poor, and she became quite dyspneic upon climbing two flights of stairs. System review was otherwise negative except for menstrual irregularity and sterility which had never been fully investigated. There was no history of orthopnea, cyanosis, or night sweats.

On physical examination the patient was noted to be a well-developed, well-nourished white female with obvious mouth-breathing but no other signs of respiratory distress. The nasal mucosa was inflamed and edematous, and there was a clear mucoid discharge. There was scarring of the tympanic membranes bilaterally. The chest was clear to percussion. However, the diaphragm descended poorly and there were fine inspiratory rales at both lung bases posteriorly. The cardiac impulse was faintly palpable at the left sternal border and the heart sounds were unremarkable. The liver was readily percussed in the left upper quadrant. The remainder of the examination was within normal limits. Sinus films showed pansinusitis, and a chest x-ray revealed complete situs inversus with left middle lobe bronchiectasis. An electrocardiogram showed changes compatible with true dextrocardia.

DISCUSSION

In this family study (Fig. 1) 29 members extending through four generations were screened by history, physical examination, and x-ray, or by history alone. Kartagener's Triad appeared in two of five siblings (see case reports). The parents, 3 siblings, 1 paternal uncle, 2 nephews, and 1 niece showed no stigmata of the syndrome by history, physical examination, and x-ray. The remaining family members were thought not to have the triad on the basis of the history alone. A reliable history is sufficient to rule out the presence of Kartagener's Triad in most individuals because of the chronicity and specificity of the symptoms. In the vast majority of cases, patients with the triad seek medical assistance because of respiratory complaints arising from sinusitis or bronchiectasis. These symptoms usually appear in childhood and are persistent. In Bergstrom's review 90 per cent of the cases had severe respiratory complaints before the

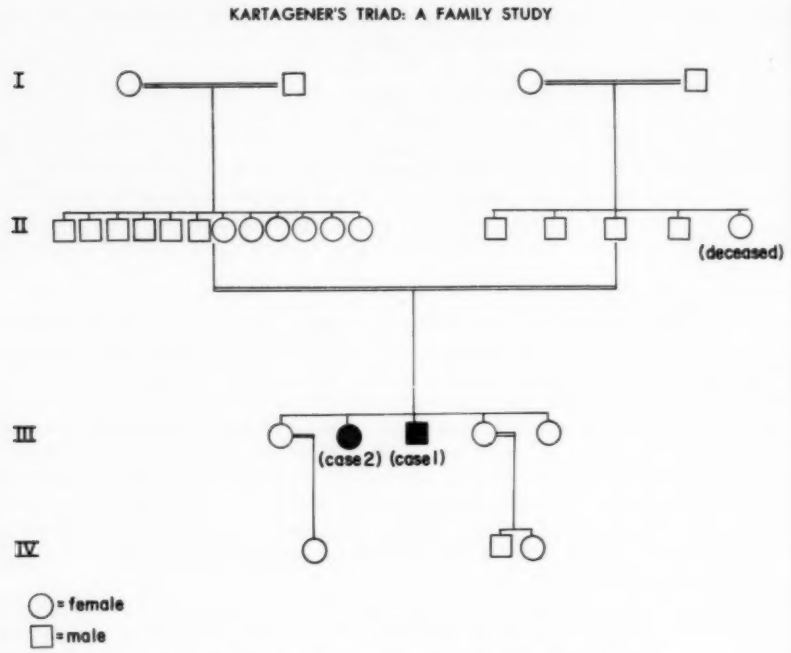

FIG. 1. Family study of the 2 cases reported here. See Discussion.

age of 14. In the 38 cases reported since 1950 (2, 4–9, 11, 12, 15, 16, 18–24, 26–28) there were only two instances in which respiratory symptoms were not present by the end of the third decade of life (4, 6).

In each of the 6 case reports since 1950 in which the familial incidence of Kartagener's Triad has been noted, only siblings were found to be involved. There is no reported instance in which the parents were found to have Kartagener's Triad. One of Karani's cases had a normal offspring (12). When all of the reported cases are considered (approximately 125), 23 are noted to have occurred among siblings. Apparently Kartagener's Triad has a high familial incidence but appears only in one generation. This pattern of familial occurrence suggests that during embryonic development some environmental factor is present which is responsible for the appearance of the triad. If a genetic factor were at fault one might expect to see the syndrome in more than one generation. This conclusion is also considered by Bergstrom.

It is also of interest to note that Kartagener's Triad is unusual in the negro race. In Bergstrom's review only one such case is mentioned, that of a twenty year old colored female reported by Adams and Churchill (1). In the 38 cases since 1950 and in 4 unreported cases from The Johns Hopkins Hospital there

were no instances of the triad in negroes; however, in some studies no mention of race is made.

SUMMARY

Two cases of Kartagener's Triad occurring among siblings are presented together with a detailed study concerning the familial incidence of this syndrome. Its rarity among negroes is noted. It is suggested that an environmental rather than a genetic factor may account for the appearance of the syndrome.

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CLINICAL MANIFESTATIONS OF HEMOGLOBIN C DISORDERS[1]

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INTRODUCTION

Hemoglobin C was discovered by Itano and Neel (1). When inherited in combination with sickle (S) hemoglobin a hemolytic disorder (S-C disease) is produced. When present in the homozygous state a disease occurs (hemoglobin C disease) with certain clinical and laboratory features which distinguish it from hemolytic disorders produced by the inheritance of other abnormal hemoglobins. Hunt and Ingram have demonstrated substitutions of single amino acids in the hemoglobin protein of hemoglobins S and C which differ from normal (2). The physical differences of the hemoglobins and the clinical disorders are presumably due to these molecular changes.

The clinical features of S-C disease have been reported, and the findings in our laboratories were recently summarized (3). Forty-three patients with this disorder have been observed in our clinics.

The features of homozygous hemoglobin C disease have been described frequently. Nine patients with this disorder have been observed in our clinics. When the clinical and laboratory features of these 9 are considered with the features of 26 reported cases distinct patterns emerge.

It has been postulated that hemoglobins A, S, and C are allelic. Family studies provide additional evidence of the allelic nature of the genes which determine these hemoglobins.

CASE REPORTS

Hemoglobin C Disease

Case 1—D.C., a 53 year old Jamaican negro was admitted to the United States Public Health Hospital in Baltimore in 1956 because of recurrent severe cramping abdominal pain. As a child he had experienced similar episodes of pain, colicky in nature, deep in the epigastrium, and often accompanied by vomiting. He then became asymptomatic for 20 years, but at age 40 abdominal pain recurred and led to the discovery of an enlarged spleen. Recurrent splenic infarction was suspected as cause of the pain and the spleen was removed. No effect on his clinical course was observed.

On physical examination he did not appear ill. He was tall and stocky. The epigastrium

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TABLE I

Thirty-five Cases of Hemoglobin C Disease Including Nine from the J.H.H.

Case	Author	Age	Sex and Race	Clinical Manifestations				Splenomegaly	Bilirubin (mg.%)	Hematocrit %	Hemoglobin grm. %	Reticulocytes %	Blood Smear				WBC (thousands per ml.)	Platelets (thousands per ml.)	RBC Fragility		Bone Marrow (E.H. = erythroid hyperplasia)	RBC Survival (½ life)	Comments
				Joint pain	Hemorrhagic manifestations	Abdominal pain	Number and morbidity with pregnancy						Target cells %	Nucleated RBC (per 100 WBC)	RBC indices (MCV/MCH/MCHC) Description	Hemoglobin crystals			Osmotic	Mechanical			
1.	D.C.	53	CM			+		+		37	10	5	30	12*	78/28/30 microspherocytes	yes*	14	250	decreased	6–9	E.H.	26 days Cr^{51}	Splenectomy 20 yrs. ago. Recurrent abdominal pain persists.
2.	M.T.-BCH 206874	26	CF				6-normal	+	1.8	27	9.5	5	20	2	77/25/34 microspherocytes	yes	4.3	67	decreased				Severe anemia with pregnancy, but no difficulty.
3.	M.E.-BCH 162443	19	CF				3-normal	+		27–39	10	1	10	1	78/28/32 microspherocytes	yes	4.5	N	decreased				Severe anemia with pregnancy, but no difficulty.
4.	V.B.-JHH 562533	25	CF		+		2-anemic	+		25–28	7	3	30	2	55/16/28 microspherocytes	yes	10	234	decreased	5–5	E.H.		Pulmonary tuberculosis with recovery. Constant anemia.
5.	G.T.-JHH A-91832	6	CF			+		+		32	10.5	4	70	3	66/22/23 microspherocytes	yes	8.9	550	decreased				Well but for recurrent abdominal pain.
6.	L.Y.-JHH 224390	30	CF				2	+		33	11	7	70	3	79/27/33 microspherocytes	yes	9.5	189	decreased	6–12	E.H.		Severe anxiety neurosis, severe head pain. Macrocytic anemia with pregnancy.
7.	E.C.-JHH 354288	31	CF				2	+		36		7	40	3	77/25/33 microspherocytes	yes	9	46–250	decreased	13–11	E.H. megalob.	17 days Cr^{51}	Megaloblastic anemia with pregnancy, asymptomatic since.
8.	C.T.-JHH 608425	20	CF	+		+		+		31	10	1	60	2	67/22/32 microspherocytes	yes	9	243	decreased	1–18	E.H.		Convulsions and polydactylism, not linked with hemoglobin C. Presumed father, A hemoglobin.
9.	W.W.-JHH 703408	48	CM					+		17–31	5.8	6–3	50	1	106/36/34, 78/28/33 micro-		10	45–250	decreased	8–12	E.H. mega-		Pernicious anemia. Following B_{12} therapy, patient asymptomatic.
	al. (4)													many*	& microcytes								WBC, Ht. and platelets. Many NRBC and rods appeared.
11.	Wheby, et	74	CM					+	0.9	31	12		mod		macrocytes								

														spherocytes							
9.	W.W.-JHH 703408	48	CM				+		17–31	5.8	6–3	50	1	106/36/34, 78/28/33 microspherocytes		10	45–250	decreased	8–12	E.H. megaloblastic		Pernicious anemia. Following B_{12} therapy, patient asymptomatic.
	al. (4)												many*	& microcytes							WBC, Ht. and platelets. Many NRBC and rods appeared.
11.	Wheby, et al. (4)	74	CM				+	0.9	31	12		mod		macrocytes & microcytes							
12.	Ranney, et al. (37)	35	CF	+			4-normal		1.2	31	10	3.3	100-many			10.4	200	decreased	9–15	E.H.	
13.	Thomas, et al. (35)	28	CF	+	+		anemic	+	1.1	35						9.6	186			N	42 days (Ashby)	Hematuria. Fall of Hct. with pregnancy.
14.	Singer, et al. (38)	22	CM					+	0.8	42	12.5	4.5	41	0	normal indices		7.6		decreased			
15.	Singer, et al. (38)	29	CM					++	2.0	41	14.5	8.8	76	25*	normal indices		9.8		decreased		E.H.		Splenectomy with no change in blood values. NRBC appeared.
16.	Singer, et al. (38)	11	CF			+		+	1.2		10.3	4.4	39	0	normal indices		8.8		decreased		E.H.		
17.	Singer, et al. (38)	9	CF					+	0.9	32	11.5	6.2	40	0	70/28/36		6.7		decreased			Mean 14 days (Ashby)	
18.	Hartz, et al. (6)	57	CM					++		34		2.6	100	1-many*	60/21/35			normal	decreased		E.H.		Splenectomy followed by appearance of many NRBC.
19.	Hartz, et al. (6)	40	CM		+			+		34		3.6	30	0	75/25/34				decreased				
20.	Hartz, et al. (6)	38	CM			+		+		37		1.	many		65/24/37				decreased				
21.	Hartz, et al. (6)	24	CF					+		34		5	many		92/28/31				decreased				
22.	Hartz, et al. (6)	37	CM					+			8.	5											
23.	Hartz, et al. (6)	39	CF					+			8.6												
24.	Spaet, et al. (39)	4	CM		+			+		30	9.8	0.8	many	some			14	180	decreased		E.H.	55 days (Ashby)	Temporal hematoma.
25.	Orvis, et al. (40)		CF	+		+		+		34	9	1.5	many						decreased		E.H.		
26.	Diggs, et al. (5)	14	WM			+		+	1.6	37	12		30	many*	71/27/38	many*	7.6		decreased				Splenectomy followed by return toward normal. Many rods and reticulocytes appeared.
27.	Levin, et al. (41)	38	CF				3	+			9.6	1	mod.								E.H.		
28.	Terry, et al. (34)	44	CF	+			2	+		31	11.2	2	30	occ.	66/24/36		4.5	123	decreased	9	E.H.	42 days (Ashby)	
29.	Morgan, et al. (42)	24	CF	+				+		33			75		microspherocytes								
30.	Lewis, et al. (11)	23	WM	+	+			+	1.2	44	14.5	1.8	50		88/18/33		22		decreased				Hematuria.

* Results following splenectomy.

TABLE I—*Continued*

Case	Author	Age	Sex and Race	Clinical Manifestations: Joint pain	Hemorrhagic manifestations	Abdominal pain	Number and morbidity with pregnancy	Splenomegaly	Bilirubin (mg.%)	Hematocrit %	Hemoglobin	Reticulocytes %	Blood Smear: Target cells %	Nucleated RBC (per 100 WBC)	RBC indices (MCV/MCH/MCHC) Description	Hemoglobin crystals	WBC (thousands per ml.)	Platelets (thousands per ml.)	RBC Fragility: Osmotic	Mechanical	Bone Marrow (E.H. = erythroid hyperplasia)	RBC Survival (½ life)	Comments
31.	Gens (43)	22	CM					++	0.8	29	10.5	12	50		anisocytosis, polychromatophilia				decreased				No symptoms.
32.	Ransome & Lang (44)	79	CM	+				+	2.3	19	6.5	2.1	pres.	2	78/25/32		31 –7.6	490	decreased		E.H.	50% at 19 days	Gout, gallstones.
33.	Tanaka & Clifford (36)	67	CF					+	5.6 –1.7	29	8.7	1.2 –4.8	30		85/29/34		10	112		inc.		50% in 21 days	Eleven pregnancies
34.	Tanaka & Clifford (36)	28	CF					++	8.8	34	10.3	2.1	90	1	58/18/30		8.7	250	decreased		E.H.		Hepatitis
35.	Tanaka & Clifford (36)	32	CF			+		++	1.8	36 –40	10–12	.6–40			93/27/28		7	73–193	decreased	N	E.H.		
	Six Cases of Hemoglobin C-Thalassemia Disease Including One from the J.H.H.																						
1.	Singer, et al. (45)	68	CF						0.9	36	12.7	3.5	43		59/21/35								77% Hemoglobin C.
2.	Singer, et al. (45)	29	CF						0.6	34	11.5	2	47		60/20/34								74% Hemoglobin C.
3.	Zuelzer, et al. (46)	6	CM							31		6.8	20		64/14/22 microspherocytes						E.H.		29% Hemoglobin C.
4.	Erlandson, et al. (9)	10	WF					+	0.4			3.6	30		60/20/34				decreased				93% Hemoglobin C.
5.	Erlandson, et al. (9)								1.8	34		3	30		63/20/32				decreased				90% Hemoglobin C.
6.	L.T.-JHH B 11332	3	CM							25	6.7	4	25		50/13/27		12	254	decreased	9–12			85% Hemoglobin C, 2% F.

was tender upon palpation and a well healed splenectomy scar was present. Physical findings were not otherwise remarkable.

Hematologic values are given in Table I. Mild anemia and chemical jaundice were present. On blood smears the erythrocytes were moderately hypochromic and polychromatophilic. There were 12 nucleated erythrocytes per 100 white cells and 30 per cent target cells, many of which had an extremely large cell diameter. Some of the erythrocytes were extremely small spherocytes. One or two rod-shaped erythrocytes were present in each high-power field. During hospitalization, gastrointestinal roentgenograms failed to reveal any cause for the abdominal pain. Roentgenograms of the bones revealed no abnormalities. Electrophoresis of the hemoglobin revealed only C hemoglobin. Osmotic fragility of the erythrocytes was decreased. The erythrocyte survival studies using Cr^{51} tagging revealed a one-half life of 26 days, a normal value. The patient was seen one year later, following an uneventful boat trip around the world, and was essentially unchanged.

Case 2—M.T., (BCH 206874), a 26 year old negro woman was admitted to the obstetrical service of the Baltimore City Hospitals in 1956 because of severe anemia complicating pregnancy. A history of previous joint, bone or abdominal pain was not elicited. Five previous pregnancies were considered by the patient to have been uneventful. Hospital records reveal, however, that at the time of the first and third deliveries, the hematocrit was 30 per cent. Physical examination revealed pallor, an easily palpable spleen at the left costal margin, and an intra-uterine pregnancy of approximately seven months duration.

Hematologic values are given in Table I. Anemia, thrombocytopenia and leucopenia were present, and there was slight chemical jaundice. Blood smears revealed many target cells and a large number of very small spherocytes. Platelets appeared to be decreased on the smear and many were unusually large. Two nucleated erythrocytes per 100 white cells were found. Bone marrow aspiration revealed that 95 per cent of the nucleated cells were erythroid elements. Megakaryocytes were present in normal numbers and appeared to be producing platelets. Electrophoretic study of the hemoglobin of this patient revealed only hemoglobin C. Two units of whole blood were administered and she was discharged from the hospital and was subsequently delivered of the pregnancy in another hospital. This patient has twelve siblings who are living and well.

Case 3—M.E., (BCH 162443), a 19 year old negro woman was admitted to the Baltimore City Hospitals in 1956 to be delivered of her third pregnancy. During her first pregnancy, in 1951, the hematocrit value was 39 per cent on one occasion and during the second pregnancy, in 1953, the hematocrit value was 30 per cent. She had experienced no previous joint, bone or abdominal pain.

Physical examination revealed no significant abnormalities except an enlarged spleen easily palpated 2 cm. below the left costal margin. There was a near term intra-uterine pregnancy.

Hematologic values are given in Table I. The blood smear revealed approximately 10 per cent target cells and an equal number of unusually small spherocytes. The platelets appeared to be present in normal numbers. Electrophoretic study of the hemoglobin revealed only hemoglobin C.

Delivery of a viable infant at term was uneventful. Studies of other members of her family (Family #19, Fig. 1) revealed that 2 of 7 siblings had homozygous hemoglobin C disease which had been virtually asymptomatic, and that 2 had hemoglobin C trait. One of the sisters with hemoglobin C trait married a man with sickle trait. Four children resulted from this union. One had S-C disease, one hemoglobin C trait, one sickle trait, and one was normal.

Case 4—V.B., (JHH 562633), a 25 year old negro woman was referred to the hematology

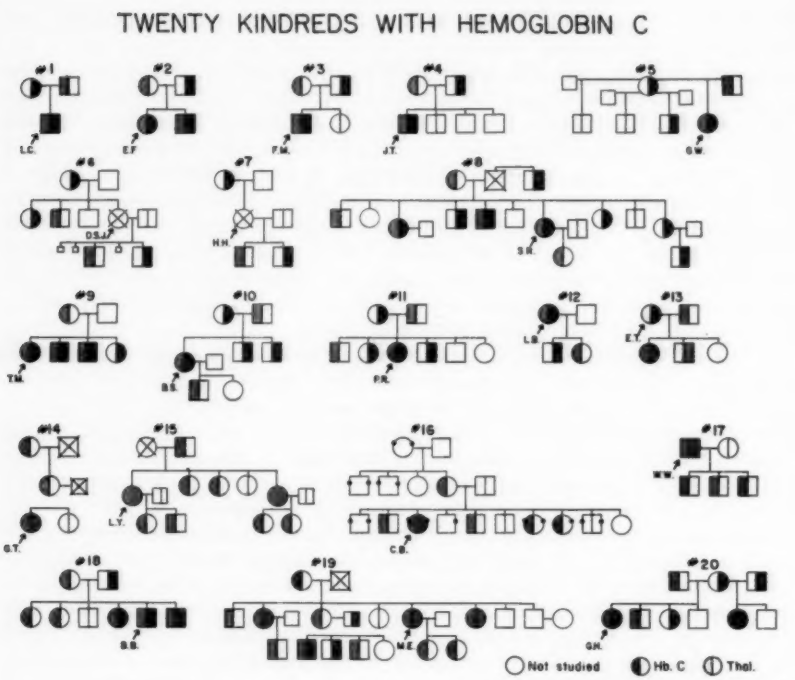

FIG. 1. The inheritance of hemoglobin C in 20 kindreds is plotted. In families #1–12, #18 and #20 the propositus had sickle cell-hemoglobin C disease. In #14–17 and #19 hemoglobin C disease, and in #13 hemoglobin C-thalassemia. See text.

clinic of the Johns Hopkins Hospital for evaluation of microcytic anemia in 1954. She had received chemotherapy, prolonged hospitalization, and resection of the upper lobe of the left lung because of tuberculosis 3 years previously. Subsequent to the pulmonary surgery she had two normal pregnancies with no apparent difficulties. Hematocrit values between 24 and 28 per cent had been recorded during previous visits.

Physical examination revealed a well developed, well nourished woman with a well healed left thoractomy scar. Moderate pallor was noted. The spleen was easily palpable at the left costal margin.

Hematologic values are given in Table I. A severe degree of anemia was present. Blood smears revealed many target cells, a moderate degree of anisocytosis and polychromatophilia. Many very small spherocytes were seen. Platelets were very large and abnormal in appearance. An occasional rod-shaped erythrocyte was seen. Electrophoretic study of the hemoglobin revealed only hemoglobin C. Osmotic fragility of the erythrocytes was reduced. The patient was given therapy with iron from a period of approximately three months without any increase in the hematocrit.

Case 5—G.T., (JHH A 91832), a six year old negro girl was referred to the Outpatient Department of the Harriet Lane Home of the Johns Hopkins Hospital in 1954 for investiga-

tion of recurrent abdominal pains of approximately three years duration. Her birth and development had been normal. The abdominal pains had been cramping in nature, were periumbilical and were frequently associated with nausea and vomiting. On several occasions intestinal obstruction had been considered a likely diagnosis.

Physical examination during an asymptomatic interval revealed a well developed child with slight pallor of the mucous membranes. The spleen was easily palpable at the left costal margin. The examination was not otherwise remarkable.

Hematologic values are given in Table I. A moderate degree of anemia was present. Blood smears revealed many target cells, a moderate degree of anisocytosis and polychromatophilia. There were three nucleated red cells per 100 white cells counted. Many very small spherocytic cells were seen. The platelets were increased in number and many were very large. Several rod-shaped erythrocytes which appeared to contain crystallized hemoglobin were seen. Roentgenograms of the chest, skull and long bones revealed no skeletal abnormalities.

Electrophoretic study of the hemoglobin of this patient revealed only hemoglobin C. Osmotic fragility of the erythrocytes was decreased. The mother, father and the maternal grandmother of the child were found to have hemoglobin C trait. The one sibling of the patient had normal hemoglobin and a normal blood smear (Family #14, Fig. 1).

Case 6—L.Y., (JHH 224390), a 30 year old negro woman attended the outpatient clinics of the Johns Hopkins Hospital in 1955 because of recurrent frontal headaches of two years duration. No history of previous illness was elicited. Normal pregnancies in 1951 and 1954 had been terminated by Caesarean section at term because of a contracted pelvis.

Physical examination revealed slight pallor of the mucous membranes and an easily palpable spleen. Hematologic studies are given in Table I. Moderate anemia was present. Blood smears contained many target cells, a moderate degree of anisocytosis and polychromatophilia. Three nucleated red cells per 100 white cells were seen. Approximately 10 per cent of the erythrocytes were exceptionally small spherocytes. An occasional rod-shaped erythrocyte could be found on some of the blood smears from this patient. The roentgenogram of the chest was normal.

Electrophoresis of the hemoglobin revealed only hemoglobin C. Osmotic fragility of the erythrocytes was decreased. The patient returned to the Prenatal Clinic in March 1958 in the 21st week of her third pregnancy. The hematocrit value was 30 per cent and iron therapy was begun. She was admitted to the hospital in the 36th week of gestation and a Caesarean section was performed. Preoperatively the hematocrit value was 21 per cent and whole blood transfusions were administered. On the first postoperative day vaginal and rectal bleeding and epistaxis occurred. The platelet count was 40,000 per ml. and on the following day was 39,000 per ml. The hematocrit value was 22 per cent and the white cell count was 6,000 per ml. The erythrocytes were macrocytic on smears. Bone marrow aspiration was not permitted by the patient. On the fifth postoperative day the hematocrit was 23 per cent and folic acid therapy with 15 mg. of folic acid per day was begun. After three days of therapy, the eighth postpartum day, the hematocrit value was 30 per cent, the white cell count was 21,000 per ml. and the platelet count was 332,000 per ml. The following day the hematocrit value was 34 per cent. She has remained clinically well since.

The father, two of the sisters and two children of this patient had the electrophoretic pattern of hemoglobin C trait and examination of the blood smears revealed increased numbers of target cells. A third sister of the patient had hemoglobin C disease (Case 7). The father of this patient stated that he had Japanese and Filipino ancestors. The mother is not living at the present time (#15, Fig. 1).

Case 7—E.C., (JHH 354288), a 31 year old negro woman was found to have hemoglobin C disease in 1955 after it was discovered that her sister had the same disorder (Case 6). She

had been hospitalized at age 14 because of pulmonary tuberculosis. At age 24 she had pelvic inflammatory disease at which time the hematocrit value was 35 per cent. At age 28 a normal pregnancy was terminated at term by Caesarean section because of a contracted pelvis. The hematocrit value at that time was 33 per cent. During the first trimester of her second pregnancy, the hematocrit value was 35 per cent. She did not attend prenatal clinic until exertional dyspnea developed in 1956 at the end of seven months of gestation of her third pregnancy.

Physical examination at that time revealed marked pallor of the mucous membranes and the presence of an intra-uterine pregnancy of approximately seven months duration. Hematologic values are given in Table I. There was a severe degree of anemia and thrombocytopenia. The blood smear revealed many target cells, a moderate degree of achromia, anisocytosis and polychromatophilia. An occasional rod-shaped erythrocyte was encountered on some of the blood smears. Many small spherocytes were present. Many of the platelets, which were present in reduced numbers, were malformed and large. There was normal gastric acidity following stimulation with histamine. The roentgenogram of the chest revealed a calcified lesion in the left hilar region. Sternal marrow aspiration revealed a moderate degree of erythroid hyperplasia and the presence of many large metamyelocytes and megaloblasts. Because of the severity of the anemia, 3,000 ml. of whole blood were administered during the next 6 days, and oral folic acid was administered in daily dosage of 15 milligrams. The hematocrit value was increased from 13 to 34 per cent during this interval, the platelet count became normal and reticulocytes appeared in the blood. At the end of the first hospital week spontaneous labor began and a viable male infant was delivered by Caesarean section. The hematocrit value increased to 37 per cent during the next week and the reticulocytes decreased to 1 per cent. Three months later the hematocrit value was 36 and the reticulocyte count 7 per cent. For the first time the spleen was palpable.

Electrophoresis of the hemoglobin revealed only hemoglobin C. The cord blood of the infant contained approximately 80 per cent fetal hemoglobin and approximately 20 per cent hemoglobin C. Osmotic fragility of the mother's red cells was decreased. Survival of erythrocytes was determined with Cr^{51} tagging and revealed a half life of approximately 17 days.

Case 8—C.T., (JHH 668425), a 20 year old negro female attended the clinics of the Johns Hopkins Hospital in 1955 because of lower abdominal pain. Frequent generalized convulsive seizures had occurred during childhood with no demonstrable cause. At 13 years of age she began to experience recurrent episodes of moderately severe aching pain in the lower extremities. The pain was not localized to the joints and was not associated with acute inflammatory changes. During the past three years she had experienced recurrent episodes of lower abdominal pain thought to be due to chronic pelvic inflammatory disease.

Physical examination revealed no abnormalities but for an easily palpable spleen and for small scars at the base of each little finger where supernumerary fingers had been ligated and removed. Hematologic values are given in Table I. A moderately severe anemia was present. The blood smear was characterized by the presence of many target cells and a moderate degree of anisocytosis and polychromatophilia. Many extremely small spherocytes were present. Many of the platelets were large and had an abnormal appearance. Roentgenogram of the chest revealed no abnormalities. Osmotic fragility of the erythrocytes was decreased and the mechanical fragility was slightly increased. Erythrocyte survival, determined by the use of Cr^{51} tagging revealed an apparent one-half survival time of twenty days which is considered to be shorter than normal. Electrophoresis of the hemoglobin revealed only hemoglobin C.

The mother, one maternal uncle and a brother of the patient had experienced recurrent generalized convulsions during childhood. The maternal grandmother, one maternal uncle

and five of the ten siblings of the patient had polydactylism with six fingers on one or both hands (#16, Fig. 1). The mother and two of the five siblings tested had hemoglobin C trait. The three siblings tested who did not have the hemoglobin C trait had normal blood smears in addition to the normal electrophoretic pattern. The father of the patient had a normal blood smear and normal hemoglobin electrophoretic pattern. It was not possible on the basis of ABO, Rh, MN, Kell and Duffy blood typing to exclude paternity. The father died suddenly before more complete studies were possible.

Case 9—W.W., (JHH 703408), a 48 year old negro man was admitted to the Johns Hopkins Hospital because of progressive weakness, dizziness, a sore tongue and epigastric distress of four months duration. He had been well previously. At the time of admission there was extreme pallor, and gray hair. There was papillary atrophy and redness of the margins of the tongue. The spleen was palpable four centimeters below the left costal margin. No neurologic abnormalities were found. Hematologic values are given in Table I. The serologic test for syphilis was negative. A severe anemia, thrombocytopenia, macrocytosis, anisocytosis and polychromatophilia were present. Many very small spherocytes were seen. Aspiration of sternal marrow revealed many giant metamyelocytes and typical megaloblasts. No free acid was present in the gastric secretions following stimulation with histamine. There was slight chemical jaundice. Gastrointestinal roentgenograms revealed a small polyp in the antrum of the stomach. Electrophoresis of the hemoglobin revealed approximately 85 per cent hemoglobin C. The remainder had the electrophoretic mobility of normal hemoglobin, but 15 per cent of the total was found to be fetal hemoglobin by alkali denaturation. It was believed that the patient had Addisonian pernicious anemia in addition to either hemoglobin C disease or hemoglobin C-thalassemia.

Following an oral dose of 5 milligrams of vitamin B_{12}, the atrophic changes and the tenderness of the tongue rapidly disappeared as did the epigastric distress. A maximal reticulocyte response of 21 per cent was attained on the third day after treatment and the hematocrit value increased steadily from 17 to 28 per cent (Fig. 2). A gastrotomy was performed and a

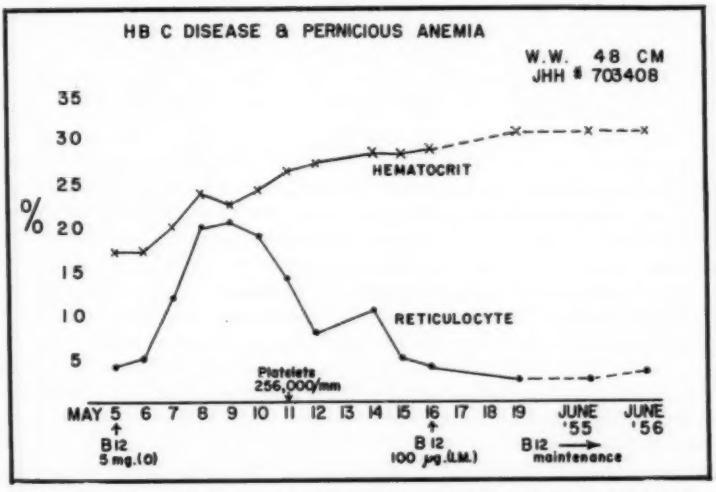

FIG. 2. Following an initial response to vitamin B_{12}, the hematocrit value of this patient (case 9) leveled off at 32 per cent.

benign polyp was removed from the stomach without postoperative complications. The reticulocyte count returned to near normal levels and 100 micrograms of vitamin B_{12} were administered intramuscularly each week. The maximal hematocrit value achieved was 32 per cent and this value has persisted to the present. The proportion of fetal hemoglobin did not decrease following treatment of the pernicious anemia. Osmotic fragility before and after vitamin B_{12} therapy was decreased. The mechanical fragility of the erythrocytes was normal. A vitamin B_{12} absorption test was followed by excretion of less than 2 per cent of the administered dose in the urine, a finding which was compatible with the diagnosis of pernicious anemia. The parents and siblings of the patient are deceased. Electrophoretic studies were performed on the hemoglobin of his two children and the blood smears were examined. Each had typical findings of hemoglobin C trait.

Hemoglobin C-thalassemia Disease

Case 10—L.T., (JHH B 11332), a negro boy was born in January 1954 and attended the Outpatient Department of the Harriet Lane Home of the Johns Hopkins Hospital on several occasions during the next two years because of frequent upper respiratory infections and bronchitis. Birth and development were normal. At age two he appeared very well developed and had only slight pharyngeal irritation. The spleen was not palpable and did not become palpable during the next year. Hematologic studies are given in Table I. Moderately severe microcytic anemia was present. The blood smears were characterized by a marked degree of achromia, anisocytosis and poikilocytosis. Approximately 25 per cent of the cells were target cells and an equal number were small spherocytes. The osmotic fragility tests revealed an increase in fragility of approximately 10 per cent of the cells, but increased resistance of the remainder. Electrophoretic studies of the hemoglobin revealed the presence of approximately 15 per cent of a compound which migrated with the mobility of normal hemoglobin, of which approximately 2 per cent was fetal hemoglobin. The remainder of the hemoglobin had the mobility of hemoglobin C.

The mother who had been well before becoming pregnant with the patient, became severely anemic during the last trimester of her pregnancy and was found to have sickle cell-thalassemia disease (#13, Fig. 1). The father had hemoglobin C trait. A sister of the patient had sickle cell-hemoglobin C disease. She had been well. The mother was included among a group of individuals with sickle-thalassemia previously reported (3).

Sickle cell-hemoglobin C Disease

Case 11—G.H., (JHH 379041), a 40 year old negro woman was admitted to the obstetrical service of the Johns Hopkins Hospital in September 1957 complaining of constant pain in the back and legs. She was in the ninth month of pregnancy and had appeared several times in the accident room of the hospital during this pregnancy complaining of pains in the limbs, back and chest. The family history was significant because the mother had sickle cell trait, the father hemoglobin C trait, and the siblings had sickle or hemoglobin C trait (#20, Fig. 1). The past history revealed that she had often complained of pains in the limbs and chest and had first been seen in the hospital at the age of 6 because of pain in her right hip. During her first pregnancy in 1954, the hematocrit value was 34.1 per cent. This pregnancy ended with a spontaneous abortion as did another later that year. In June of 1957 the hematocrit value was 29.5 per cent. She received iron and her hematocrit value rose to 34.5 per cent. She had been treated with penicillin for latent syphilis because of a positive serologic test for syphilis, but a treponema immobilization test was negative.

Physical examination on admission revealed that her temperature was 99.2 F., pulse 92,

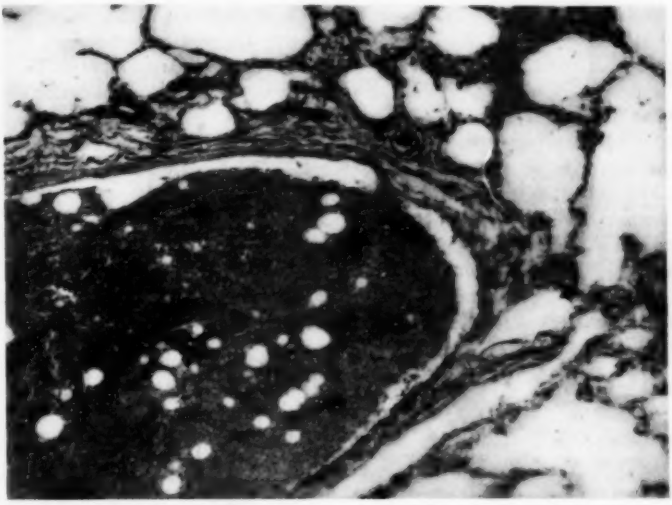

FIG. 3. An embolus of bone marrow is present in a pulmonary vessel (× 40). From a fatal case of sickle cell-hemoglobin C disease.

blood pressure 125/60. She was dehydrated. The fetal heart was audible. Her back pain disappeared but rales and rhonchi were heard in the right chest. Parenteral penicillin therapy was begun. There was a moderate degree of anemia, jaundice and leucocytosis. She became tachypneic, her heart rate increased, she developed profuse sweating and the blood pressure rose to 150/90. The rhonchi increased and audible inspiratory and expiratory wheezes were evident. Electrophoresis of the hemoglobin revealed sickle and C hemoglobins. Parenteral penicillin, aminophyllin, adrenalin, and hydrocortisone were administered. There was no improvement. She received oxygen and digitalis, but died 37 hours after admission. At autopsy focal infarctions were found in the bone marrow and massive bone marrow emboli were found in the lungs (Fig. 3). Fat emboli were seen in the brain and kidneys.

Case 12—E.J., (JHH 787170), a 30 year old negro woman was admitted to the Johns Hopkins Hospital in October, 1957, because of unusual retinal vascular changes in the right eye. A sister of the patient was said to have anemia and trouble with her eyes. The patient had experienced recurrent pain in the legs since childhood. Recurrent episodes of inflammatory change in the left eye began in 1951 and led to blindness by 1956. The eye was subsequently enucleated. In 1957 a melanoma of the right optic nerve was observed, and tortuous vascular loops were noted in the periphery of the fundus. A clinical diagnosis of sickle cell-hemoglobin C disease was made by Dr. A. E. Maumenee and substantiated by electrophoretic study of the hemoglobin. She was admitted to the hospital for diathermy to the involved retinal areas.

At the time of admission the patient was obese. The vital signs were normal. There was no icterus. The left eye was surgically absent. A black mass extending forward 6 to 8 diopters covered most of the optic disc of the right eye. Around most of the periphery of the fundus were clusters of corkscrew-like dilated and occluded vessels. The remainder of the physical examination was unremarkable. The serologic test for syphilis was negative. A mild anemia and mild thrombocytopenia were present.

The involved areas of the fundus were treated with diathermy in an attempt to prevent retinal detachment. On the third postoperative day the patient complained of dyspnea, pleuritic pain on the right, and a non-productive cough appeared. Signs of bilateral pulmonary consolidation appeared, the temperature rose to 102°F. and consolidation of the lungs increased to involve the middle as well as basilar regions. The hematocrit value fell to 30 per cent and 4 units of whole blood were administered to alleviate the anemia and to suppress the patient's production of defective erythrocytes. The hematocrit value increased to 42 per cent and the serum bilirubin to 1.8 mg. per 100 ml. On the fifth day of the illness the patient became confused and progressively disoriented. Generalized convulsive seizures developed and she died the following day.

At autopsy multiple small thrombi in the pulmonary arteries and focal areas of pulmonary necrosis were present. Bone marrow emboli were not found, but fat-laden macrophages were identified in the brain. The spleen was small and showed old areas of infarction.

RESULTS

The clinical features and laboratory data of 35 cases of hemoglobin C disease including 9 from our clinics are tabulated in Table I. The youngest patient was 4 years of age and the oldest was 79. Twenty were females. One was a Caucasian of Sicilian origin, one was of Dutch origin and the rest were negroes. In one family Chinese and Japanese ancestry was claimed and one patient stated that his mother was Spanish.

Clinical features—The presenting complaint varied widely. Among the 9 individuals in our group the presenting complaint was abdominal pain in 3, headache in 1, anemia of pregnancy in 2, pernicious anemia in 1, and chronic microcytic anemia in 1. One patient was discovered during a family study.

Poorly localized, recurrent pain which was not associated with inflammatory joint change occurred in 8 of the 35 patients. Abdominal pain was experienced by 9 members of the group. In 5 the pain was episodic and poorly localized in the epigastrium, but in 3 others it was severe enough to mimic intestinal obstruction. Convulsive seizures occurred in 2 individuals. Seizures first occurred in the sixth decade in the patient of Wheby and his associates (4) and during childhood in one from our group. Seizures occurred in 2 members of the family of this latter patient, in both of whom hemoglobin C trait was present. No cause for the convulsions became apparent and the relationship to the hemoglobin disorder is unknown. Hemorrhage manifestations were encountered in 5 of the 35 patients. One instance each of recurrent epistaxis, hemoptysis and bilateral periorbital hemorrhage and 2 of hematuria were encountered. Headache was severe enough to cause 3 of the individuals to seek medical attention. In each case the pain was generalized and remained unexplained. The habitus of the 9 patients in our group was not abnormal and no comment of abnormality was made concerning the previously reported cases. In particular, hyperextensibility of the fingers, tower skull, and unusually long extremities were not present. Jaundice was frequently noticed. Nine of the previously reported patients had clinical jaundice with a serum bilirubin value between 1.2 and

2.0 mgms. per 100 ml. In 4 patients of the present group a serum bilirubin value between 1 and 2 mgms. per cent was encountered on at least one occasion. The other individuals were not observed to have an elevated bilirubin value or an increase in the icterus index. Ocular changes were not encountered in these patients. Particular note is made of this negative finding because of the high incidence of intraocular hemorrhage or retinal vascular abnormalities in patients with sickle cell-hemoglobin C disease. Cardiac abnormalities and pulmonary disease were infrequently encountered. Recurrent episodes of chest pain or pneumonia, common manifestations of sickle cell diseases, were not observed.

The spleen was enlarged in 34 of the 35 cases. Splenectomy was performed in 5 of the patients, in 2 for suspected splenic infarction, once because of extreme enlargement of the spleen, once because of hemolytic anemia and thrombocytopenia, and once because of unexplained, recurrent abdominal pain. Microscopic examination of the spleen in the 4 reported cases revealed dilated sinusoids, few follicles and moderate to marked red cell congestion. Pigment deposition was marked in the patient of Diggs and his associates (5), but was minimal in the spleen studied by Hartz and his associates (6). Both Diggs and his associates and Wheby and his associates (4) found that many of the erythrocytes in smears from the spleens of their patients were characterized by rod-like crystals of the hemoglobin.

Renal function was not studied in detail in these patients. However, the specific gravity, protein content, and microscopic sediment of the urine were not abnormal. In the two patients with gross hematuria there was no other abnormality of renal function. Skeletal abnormalities were not observed in roentgenograms of the patients with hemoglobin C disease, 8 of whom were subjected to an extensive bone survey.

Nine of the 20 females studied underwent uncomplicated pregnancies. Two were delivered by Caesarean section because of contracted pelvis. One of these individuals developed a severe megaloblastic anemia during the last trimester. In at least 5 others the hematocrit value was substantially less during the last months of gestation than in the postpartum state. The efficacy of folic acid therapy could not be evaluated, but the megaloblastic changes disappeared following delivery in the one patient whose marrow showed this abnormality.

Laboratory studies—Many of the patients with hemoglobin C disease had anemia. Of 21 cases for which values are available 8 had an hematocrit value of 37 per cent or higher on at least one occasion. During several years of observation of the 9 patients in our group, the hematocrit value of 8 was repeatedly 32 per cent or less and in one patient was 25 per cent on repeated examinations. In most cases examination of the bone marrow revealed erythroid hyperplasia.

The erythrocytes were microcytic in 10 reported cases and in all 9 of our

cases. In 9 of the cases collected from the literature, the mean erythrocyte volume was normal. Macrocytosis was transiently present in 3 of our patients, during pregnancy in two and during an untreated stage of pernicious anemia in the other. Blood smears of patients with hemoglobin C disease demonstrated from 10 to 90 per cent target cells (Fig. 4). Some degree of anisocytosis, poikilocytosis and polychromatophilia was present in all instances. Very small, rounded and dense erythrocytes having the appearance of microspherocytes were observed in the blood smears of all 9 of our patients. This combination of red cell abnormalities was so characteristic as to suggest the diagnosis even before electrophoresis of the hemoglobin was performed. Nucleated red cells were encountered in the blood of 9 patients in whom the spleen was intact but in no instance exceeded 3 per 100 white cells. Following removal of the spleen circulating normoblasts occurred in great profusion, varying from 12 to 80 per 100 leucocytes. A reticulocyte count of greater than 1.5 per cent was encountered in 20 of 32 patients. In only 5 was there greater than a 6 per cent reticulocytosis. Following splenectomy a reticulocyte count of 87 per cent was recorded in one patient.

Rod-shaped erythrocytes with squared corners which suggested the intracellular crystallization of hemoglobin were identified on smears from 5 of our 9 patients (Fig. 4). In one patient whose spleen had been removed, rodlike erythrocytes were encountered in greatly increased numbers. These intraerythrocytic crystals were similar to those observed by Diggs (5) and by Wheby (4) in 2 patients with hemoglobin C disease following removal of the spleen. Crystal formation could be produced by gradual drying of fresh blood smears from 2 of our patients in whom the spleen was intact. Blood from the splenectomized patient developed the rod-like forms more rapidly and in greater profusion under similar circumstances. The leucocyte count was normal or slightly elevated and the differential cell counts were not remarkable in patients with hemoglobin C disease. Thrombocytopenia occurred in 8 of the patients. In 3 of these the thrombocytopenia was noted during pregnancy and the platelet count returned to normal in the postpartum period. In one the thrombocytopenia was associated with pernicious anemia in relapse and in one individual it was associated with hemolytic anemia.

Hemoglobin C trait—Eleven thousand electrophoretic studies of hemoglobin have been performed in our laboratories. Most of the blood samples were obtained from negroes. There have been 240 instances of hemoglobin C trait. This number represents 2.1 per cent of the total but may not be a true incidence percentage since to a certain extent the hemoglobin samples were selected. However, the value is similar to that found in a survey of 500 negroes in 1952 (7). One of these individuals with hemoglobin C trait had two episodes of unilateral, renal hematuria, one had a persistent, severe microcytic hypochromic anemia which did not respond to iron and in several others dental

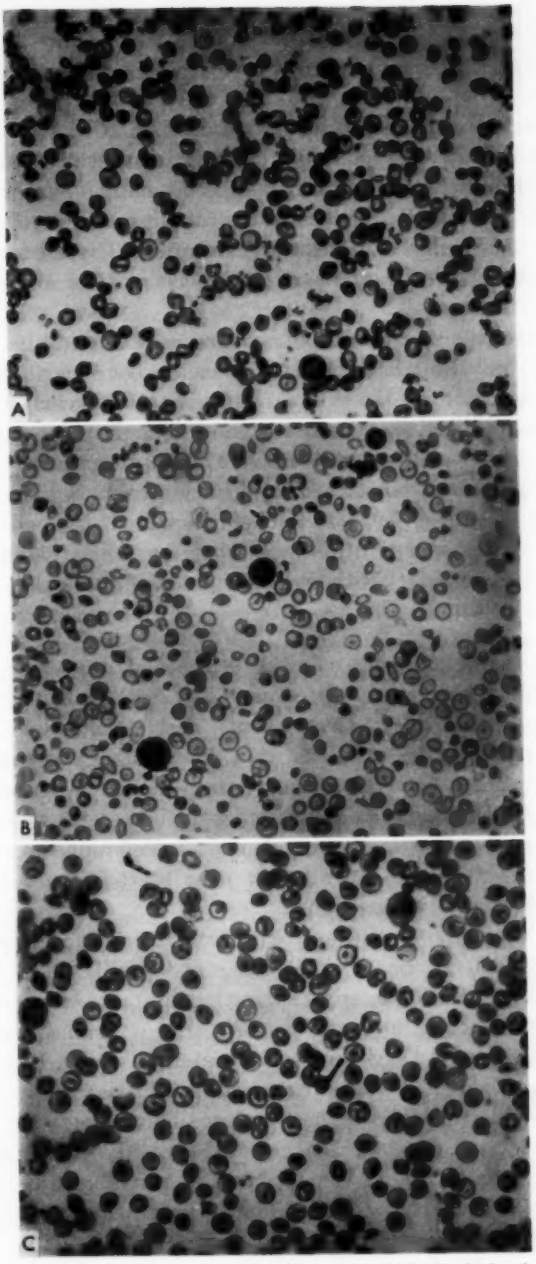

FIG. 4 a). Blood smear from hemoglobin C disease (× 500). Variation in size of erythrocytes is marked. Target cells, "folded" erythrocytes, and microspherocytes are seen. b). Blood smear from hemoglobin C-thalassemia (× 500). Hypochromia, variation in size and shape, and numbers of microspherocytes are greater than in hemoglobin C disease. c). Blood smear from hemoglobin C disease after splenectomy (× 500). Target cells are present in large numbers and one rod-shaped erythrocyte is present.

roentgenograms revealed spotty areas of bone infarction. The spleen was removed from one individual following traumatic rupture and was found to have many areas of perifollicular hemorrhage indistinguishable from those encountered in sickle cell trait. Target cells were regularly present in the blood smears, and the osmotic fragility was frequently decreased. Crystal formation within the erythrocytes could be produced in the blood from an individual with hemoglobin C trait.

Two individuals with Addisonian pernicious anemia were also found to have hemoglobin C trait. Both had a moderately enlarged spleen, an unusual feature of pernicious anemia, but the clinical course of the pernicious anemia did not appear to be altered and anemia subsided after vitamin B_{12} therapy. In another individual with hemoglobin C trait, a severe hemolytic anemia occurred shortly after the ingestion of a large amount of aminopyrine. Subsequent study revealed that the patient had genetically determined "primaquine-sensitive" erythrocytes. There seemed little doubt that the hemolytic anemia resulted from the latter abnormality without participation of hemoglobin C.

DISCUSSION

During the course of studies on sickle cell anemia, Itano and Neel (1) discovered a child whose erythrocytes contained both sickle hemoglobin and another hemoglobin which was subsequently designated as *hemoglobin C*. The father of their patient had sickle cell trait. The mother was heterozygous for normal and for hemoglobin C, a genetic state which was subsequently designated as *hemoglobin C-trait*. The disorder in the patient was designated as *sickle cell-hemoglobin C disease*. Many genetically determined abnormal hemoglobins were described subsequently but none has been encountered as frequently as sickle and C hemoglobins.

Surveys of randomly selected negroes revealed an incidence for hemoglobin C of 2 per cent in Baltimore (7) and 3 per cent (8) in Texas. Among approximately 800 white persons similarly examined, no instance of hemoglobin C was discovered. Subsequently, however, sporadic cases in non-negroes were found. The patient reported by Diggs and his associates (5) was an Italian child with homozygous hemoglobin C disease. Erlandson and his associates (9) discovered hemoglobin C-thalassemia disease in two white siblings, the father of whom was of Italian descent and who had hemoglobin C trait. Huisman, Van der Schaaf and Van der Sar encountered hemoglobin C in a white family (10). Lewis, Anderson and Baskind (11) recognized homozygous hemoglobin C disease in a white family living in Capetown, South Africa.

Recognition of this abnormal form of hemoglobin led to a better understanding of many disorders which were characterized by anemia and other hematologic manifestations. Its recognition was also of importance to geneticists who used the hemoglobin as a genetic tag which could be traced with great

accuracy. Accordingly, numerous population groups throughout the world were investigated in an attempt to determine the place of origin and paths of migration from that origin for hemoglobin C. Edington and Lehmann obtained data on the incidence of hemoglobin C in communities of the Gold Coast of Africa (12). Hemoglobin C was present in 21.6 per cent of a surveyed population in the northern region of this state and in only 9.2 per cent of the southern part of the Gold Coast. The frequency in nearby Nigeria was 7 per cent. In other regions of Africa only isolated cases were encountered or hematologic surveys revealed no hemoglobin C in the population (12, 13). In no other region surveyed was the incidence of hemoglobin C as high as in the Gold Coast. The assumption has been made and seems reasonably well supported that this location was the origin of hemoglobin C in man.

The maintenance of a gene frequency as high as 21.5 per cent in a community requires that the gene be non-lethal or that it convey some advantage to the affected individual. Allison presented evidence that a high gene frequency for hemoglobin S was possible in certain areas of Africa because the individuals heterozygous for hemoglobin S enjoyed a protection from malaria due to P. falciparum which was endemic in those regions (14). Raper confirmed these findings (15). Because hemoglobin C disease is much less severe than sickle cell anemia, a state of balanced polymorphism should be possible with less of an advantage in favor of heterozygosity for hemoglobin C than was necessary to maintain a high frequency of hemoglobin S. The studies of Edington and Laing have demonstrated that hemoglobin C does not offer protection against malaria (16) and no other protective influences have been suggested. The distribution and genetic expectancy of hemoglobin C were well reviewed by Neel and his associates (17).

Biochemical expressions of hemoglobin C are, without doubt, related to its clinical manifestations, although the exact nature of the association is not established at the present time. Itano demonstrated that hemoglobin C is more soluble in its reduced form than is normal hemoglobin (18). Sickle hemoglobin, on the other hand, is far less soluble than normal in the reduced form and the combination of S and C hemoglobins in sickle cell hemoglobin C disease has been found to be less soluble than normal. A second significant abnormality in behavior of the hemoglobin was demonstrated by Diggs and his associates (5). These workers described the production of intraerythrocytic crystals of hemoglobin C in blood smears of individuals with hemoglobin C disease and subsequently observed similar crystal formation in the erythrocytes of individuals with hemoglobin C trait. This phenomenon appears to be unique to hemoglobin C and the rigid, rod-shaped structure which is produced may be of importance in the pathogenesis of the clinical disorder.

Recently Hunt working with Ingram (2) demonstrated that hemoglobin C differs from normal by a single amino acid. In hemoglobin C a molecule of

lysine replaces a molecule of glutamic acid in a peptide of the hydrolyzed globin. The resulting increase of two charges above the state of normal hemoglobin could account for the marked difference in the electrophoretic mobility between C and normal hemoglobins. This characteristic difference is maintained on filter paper, agar gel, in the free electrophoretic cell, or on resin exchange column chromatography (19).

Muryama (20, 21) has obtained evidence that in addition to this qualitative change, the configuration of the C hemoglobin molecule differs from normal. He demonstrated that the A and C hemoglobin molecules contained eight free sulfhydryl groups. The intra-atomic distance between two pairs of sulfur atoms in the A hemoglobin was such that where mercaptan groups were formed under appropriate experimental circumstances only six molecules of mercury were bound. Under similar experimental circumstances the hemoglobin C molecule was able to bind eight atoms of mercury, a finding which was interpreted as evidence that the orientation of the sulfhydryl groups on the surface of the hemoglobin molecules differed. Other structural differences between these molecules may exist but are as yet unrecognized.

Hemoglobin C was believed to be an autosomal dominant character by its discoverers (1). Genetic data to support this hypothesis were rapidly accumulated by many workers. The clinical manifestations of the inheritance of hemoglobin C are modified by the presence of other abnormalities of the erythrocyte. The gene for hemoglobin C has incomplete expression in the heterozygous state, a greater degree of expression when some other abnormalities of the red cell are present and is presumed to have full expression in the homozygous hemoglobin C state.

The incomplete expression in the heterozygous individual results in the production of erythrocytes which contain approximately 33 per cent hemoglobin C and 67 per cent normal hemoglobin. Target cells vary from 1 to nearly 100 per cent on the blood smears and occasionally clinical manifestations suggestive of the homozygous hemoglobin C disease are encountered. Because of the presence of target cells, the osmotic fragility of the erythrocytes is often reduced and in some instances it is markedly less than normal (Fig. 5). The life span of erythrocytes of individuals with hemoglobin C trait is normal (22, 23). Clinical manifestations associated with C trait have been rare. Infarction of dental bones was noted in some individuals (24). Unilateral, renal hematuria occurred in one. The spleen of one individual with hemoglobin C trait who had died of an unrelated cause was found to contain perifollicular hemorrhages which were indistinguishable from those encountered in the spleen of individuals with sickle cell trait (25). A moderately severe microcytic, normochromic anemia was present in one individual with hemoglobin C trait. Repeated studies over a four year period did not reveal an explanation for the anemia. An association between these occurrences and the presence of hemoglobin C

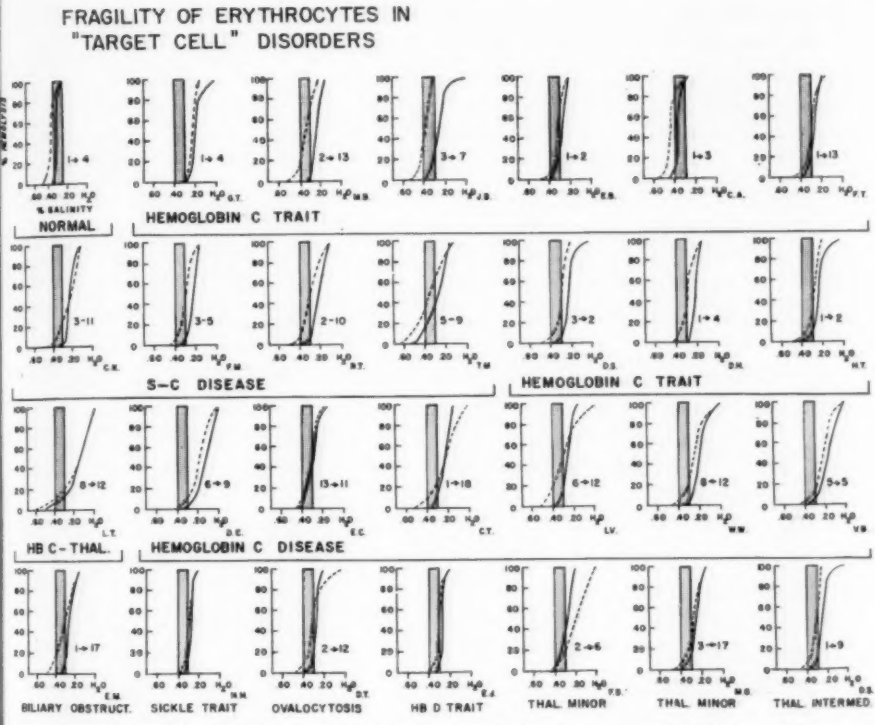

FIG. 5. Osmotic fragility is plotted before incubation (——— line) and after 24 hour incubation (---- line). Figures to the right of each curve represent percentage mechanical fragility before and after incubation. Dappled bar represents approximate range of normal non-incubated values. The displacement of most curves to the right is an indication of resistance.

in the heterozygous state can only be speculative since the observations are few, but a parallel can be drawn to the experience with sickle cell trait. Manifestations of sickle cell disease, such as bone infarction, hematuria, infarction of the spleen and hemolytic anemia (26) have been recognized with increasing frequency in individuals with sickle cell trait.

The occurrence of some other hematologic abnormalities in individuals with hemoglobin C trait was encountered when hemoglobin C trait was found in 2 individuals with Addisonian pernicious anemia, 1 with primaquine-sensitive erythrocytes, and 2 with hereditary elliptocytosis (27). There was no evidence of gene interaction or of linkage.

When hemoglobin C and certain erythrocyte abnormalities are inherited, clinical manifestations which depend upon summation of the gene effects may

TABLE II

Certain Clinical Manifestations of 43 Patients With Sickle Cell-Hemoglobin C Disease

Increased morbidity or mortality associated with pregnancy	9 of 13
Aseptic necrosis of ends of long bones	8 of 43
Intraocular hemorrhage, retinal vascular proliferation, or retinal detachment	8 of 43
Renal hematuria	3 of 43

result. In 1953 the clinical manifestations of 17 patients with sickle cell-hemoglobin C disease studied in our laboratory and of 11 cases collected from the literature were reviewed (3). Since that time a total of 43 individuals with sickle cell-hemoglobin C disease have been studied in our laboratory and data concerning many others have been reported in the literature. Aseptic necrosis of the ends of the long bones, ocular changes consisting of a peculiar form of retinal vascularization, retinal detachment, or vitreous hemorrhage, and morbidity and mortality associated with pregnancy were noted to occur with greater frequency in sickle cell-hemoglobin C disease than in other hemoglobinopathy (Table II). Hook and Cooper (28) carried out an extensive review of sickle cell anemia and sickle cell-hemoglobin C disease and reported two instances of aseptic necrosis of the femoral head of a group of 15 patients with sickle hemoglobin C disease. These authors and others encountered similar changes in patients with sickle cell anemia. Molina and Marks (29) found two cases in sickle cell anemia and two in sickle-hemoglobin C disease. This form of bone necrosis has not been encountered in hemoglobin C disease.

In our clinics aneurysmal changes in retinal vessels, sometimes with retinal detachment, were recognized in one patient without a demonstrable hematologic disorder, in none with sickle cell anemia and in eight individuals with sickle cell-hemoglobin C disease. In several instances the diagnosis of S-C disease was made by the ophthalmologists. These changes were discussed by Hannon (30) (Fig. 6). Edington found an apparently identical change in one patient with sickle cell anemia and one with sickle cell-hemoglobin C disease in Ghana (31).

Curtis (32) has reviewed the obstetrical implications of sickle cell hemoglobin C disease and sickle cell anemia in this hospital. Sickle cell anemia rarely became more severe during pregnancy and episodes of pain were seldom experienced. In sickle cell-hemoglobin C disease, however, anemia frequently increased in severity as gestation progressed and crises occurring near or following delivery were to be expected. Four maternal deaths associated with sicklemia in the Johns Hopkins Hospital in the past 20 years all occurred in sickle-hemoglobin C disease. Shelley and Curtis (33) recently reported the pathological evidences of fat embolization in fatal sicklemia. Their review of the literature emphasized the fact that sudden maternal death in other re-

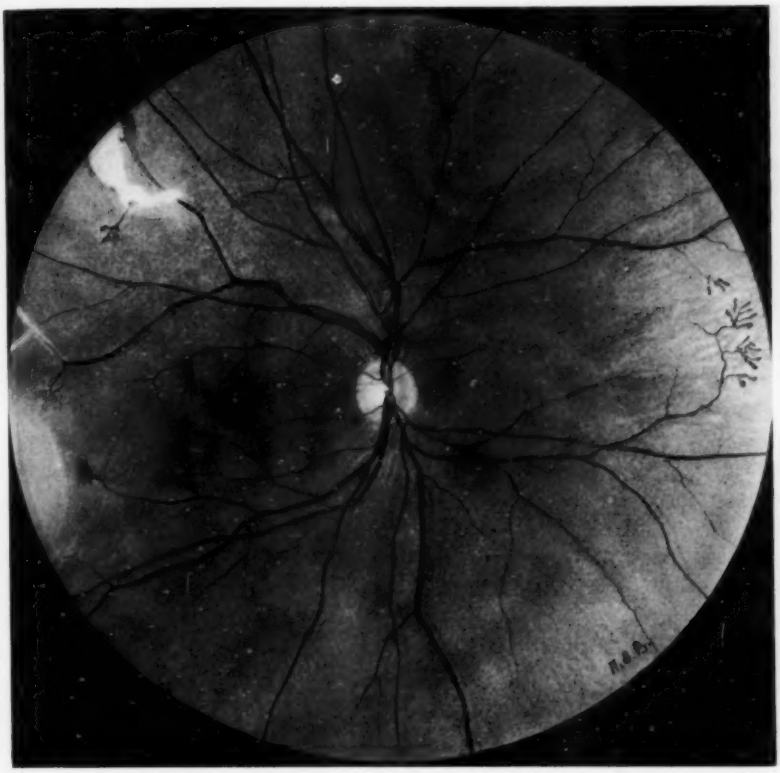

FIG. 6. Retinal vascular proliferation in sickle cell-hemoglobin C disease. (Drawing by Anette M. Burgess reproduced by permission of Am. J. Ophthalmology.)

ported cases was accompanied by clinical or pathologic evidence of bone marrow embolization and that most of the cases were clinically suggestive or electrophoretically proved instances of sickle cell-hemoglobin C disease.

The two cases of sickle cell-hemoglobin C disease described in the present report illustrate the importance of marrow and fat embolization in the pathogenesis of certain clinical manifestations of this disorder. The occurrence of severe pain in the back or extremities followed by death with evidence of embolization of the lung was observed during pregnancy in one individual and following a relatively light anesthesia for an ophthalmologic procedure in a second. These important clinical features of sickle cell-hemoglobin C disease often occur in the absence of significant degree of anemia. These clinical manifestations are encountered rarely in hemoglobin C disease, hemoglobin C-thalassemia or sickle cell anemia and the frequency with which they occur in sickle cell-hemoglobin C disease remains unexplained.

Hemoglobin C-thalassemia was encountered less often than hemoglobin C disease or sickle cell-hemoglobin C disease. The clinical manifestations include a moderate degree of anemia and mild to severe bone pain. The spleen was palpable in one of the six cases. The most characteristic feature of the disease was the appearance of the blood smears. Large, thin target cells and small, fragmentary erythrocytes predominated. In addition, target cells and microspherocytes similar to those seen in the blood in hemoglobin C disease were present in large numbers.

The clinical manifestations of the homozygous inheritance of hemoglobin C are remarkably well tolerated by most affected individuals. Hemoglobin C constitutes about 33 per cent of the hemoglobin in C-trait, 50 per cent in sickle-hemoglobin C disease, 80 per cent in C-thalassemia and is virtually the only hemoglobin present in homozygous hemoglobin C disease. Fetal hemoglobin is rarely found to be increased above normal levels in hemoglobin C disease but is regularly increased in hemoglobin C-thalassemia. One patient in the series of cases presented here (Case 9) had approximately 15 per cent fetal hemoglobin and may well have hemoglobin C-thalassemia. Because of the similarity of the clinical disorder of this patient to the other instances of hemoglobin C disease and because of the possibility that the fetal hemoglobin resulted from interaction of the hemoglobin C gene and pernicious anemia which was also present, the tentative designation of hemoglobin C disease was maintained.

A definitive genetic diagnosis of hemoglobin C disorders is often reached after investigation of other members of the kindred. The data in Figure 1 when considered with those gathered by other workers leave little doubt that the hemoglobins C, S and A are alleles and have a high order of penetrance. Furthermore, it seems probable that the locus of the thalassemia gene is closely linked to that of the CSA gene. In one kindred (#16, Fig. 1) the putative father of a patient with hemoglobin C disease had only normal hemoglobin when studied with filter paper electrophoresis. Careful hematologic study of this individual revealed no evidences of thalassemia, nor was that abnormality apparent among his ten children. It was not possible on the basis of ABO, CDE, MN, Kell or Duffy typing to exclude paternity. The possibility exists, however, that he was not the genetic father of the patient. It is also possible that hemoglobin C was inherited by the father, exhibited a small degree of penetrance and the small amount of hemoglobin C present could not be detected by the procedures used. The patient alternatively may have had an abnormality which is as yet unrecognized and which resulted in suppression of all except C hemoglobin production. This seems unlikely as does the possibility that thalassemia in the putative father was overlooked. Hemoglobin C in this patient may have been inherited from the mother and a corresponding normal

gene lost. Death of the father of the patient terminated the studies of this situation.

In most instances hemoglobin C disease is associated with few clinical manifestations. It is usually recognized during the course of investigation of unrelated medical problems. The essential benignity is reflected in the old age attained by many persons with hemoglobin C disease and the relatively small number of cases recognized during childhood. In a more serious familial hemolytic disorder, sickle cell anemia, on the other hand, clinical manifestations occur commonly in childhood and survival past age 40 is unusual.

Joint pains or episodic abdominal pains were the only symptoms experienced with regularity by patients with hemoglobin C disease. Headaches and convulsive seizures were encountered but their relationship to the hemoglobinopathy is less clear.

Increased morbidity during pregnancy was not a feature of most cases. In several instances anemia became more severe during pregnancy but was well tolerated. In two patients pancytopenia with macrocytosis occurred at term. In one of these the bone marrow was megaloblastic and marrow examination was not permitted by the other. In both patients following delivery and folic acid administration, there was a return of the blood values to the pre-pregnancy levels.

Physical abnormalities usually associated with hemoglobin C disease were pallor and splenomegaly. Pallor is readily explained by the anemia which was almost invariably present. Splenomegaly is recognized as a frequent concomitant in many hemoglobin disorders and occurred in virtually all instances of hemoglobin C disease. The possibility exists that the enlargement of the spleen in hemoglobin C disease results from the sequestration and removal from the circulation of abnormally formed erythrocytes. Sequestered cell forms might be target cells, tiny spherocytic erythrocytes or the markedly distorted forms which contain elongated hemoglobin crystals. This latter form of abnormal cell is encountered rarely in the peripheral blood of individuals with most forms of hemoglobin C disease, but may be encountered in very large numbers following removal of the spleen.

Several individuals with hemoglobin C disease were found to have leukopenia, thrombocytopenia or hemolytic anemia secondary to splenic effects. Removal of the spleen in such instances was followed by return of the blood values towards normal, but some anemia persisted in most cases. The secondary panctopenia which was encountered does not seem characteristic of hemoglobin C disease.

The characteristics of the anemia of hemoglobin C disease are not sharply defined. Some individuals were said to have a macrocytic anemia. In most cases, however, the erythrocytes were microcytic and contained a normal hemoglobin concentration. Some patients with hemoglobin C disease had a

normal hematocrit value but most had a moderate and persistent anemia. The survival of erythrocytes was normal in some but markedly shortened in other instances. Individuals with a hematocrit value as low as 30 per cent and with a shortened survival time of the erythrocytes did not have an accordingly increased excretion of bilirubin pigments or a compensatory reticulocytosis. Terry and his associates (34), Thomas and his associates (35) and Tanaka and Clifford (36) each demonstrated that the iron turnover rate in hemoglobin C disease was normal or very slightly increased. It seems evident that the causative mechanism must include the formation of imperfect small and easily destroyed erythrocytes, and an inadequate attempt by the body to compensate for the anemia.

Target cells and microspherocytes in the blood smears of individuals with hemoglobin C disease are characteristic of the disorder, but are not pathognomonic. Only small numbers of target cells were present in the blood of some individuals and in some instances of hemoglobin C trait virtually 100 per cent of the cells were target forms. However, the mixture of target forms and microspherocytes in a blood smear occurs in very few conditions other than hemoglobin C disease or hemoglobin C-thalassemia. Microspherocytes appear as irregularly rounded, dense cells with a diameter less than one third that of a normal erythrocyte (Fig. 4). Other workers have described a 'folded erythrocyte' or 'oat shaped' irregularly outlined cell in this condition. Undoubtedly these morphologic changes are associated with the abnormal hemoglobin mechanism.

Fragility of the erythrocytes is invariably abnormal in hemoglobin C disease. Resistance to hypotonic saline may be demonstrated in some cases of hemoglobin C trait and is characteristic of sickle cell-hemoglobin C disease, hemoglobin C disease and hemoglobin C-thalassemia. An increase in the osmotic resistance can be correlated with the presence of target cells in these disorders. Curiously, however, some cells become more resistant following incubation while others become unduly fragile. Mechanical fragility is generally increased. The abnormal fragilities may be associated with other morphologic changes as well.

Thrombocytopenia was recognized in 7 instances of hemoglobin C disease. It was not possible, however, to correlate the hemorrhagic manifestations which occurred with thrombocytopenia.

SUMMARY

1. The clinical manifestations of hemoglobin C trait, sickle hemoglobin C disease, hemoglobin C-thalassemia and hemoglobin C disease are discussed.

2. Nine cases of hemoglobin C disease are presented and the data concerning a total of 30 cases are collated.

3. Two cases of sickle cell-hemoglobin C disease are reported in which fat and bone marrow emboli were the cause of death.

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ABSTRACTS OF PAPERS PRESENTED BEFORE THE MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

October 13, 1958

Clinical Experiences in the Treatment of Hyperthyroidism

RADIOIODINE

WORTH B. DANIELS, JR.

From 1949 through the first six months of 1957, 210 patients with hyperthyroidism were treated with radioiodine (I-131). The quantity of radioiodine given to individual patients varied from 2.5 millicuries to 69.5 millicuries; 127 patients (60 per cent) required less than 9.0 millicuries. Multiple doses were administered in 85 patients.

One hundred and seventy-eight patients recovered and 12 definitely improved. Chief complication was the development of permanent hypothyroidism in 24 patients. One patient developed mild exophthalmos following treatment with radioiodine; two had progression of exophthalmos during therapy. No instance of thyroid cancer, leukemia, or hypoparathyroidism has occurred. One thyrotoxic patient with angina pectoris died of an acute myocardial infarction 11 days after a single dose of radioiodine.

ANTITHYROID DRUGS

ROBERT B. KATIMS

Hyperthyroidism can regularly be controlled by the administration of antithyroid drugs. During the period 1946 through 1956, a total of 287 patients with hyperthyroidism were treated in this way by the endocrine group at The Johns Hopkins Hospital. Of these, 79 patients have completed a course of long-term antithyroid drug therapy and have remained under observation for at least thirty months after cessation of medication.

Of these 79 patients, 36 (46 per cent) remained in remission for at least thirty months after cessation of medication. Of the 43 patients who had a recurrence of hyperthyroidism, 15 were given a second course of therapy. In this group 8 patients remained euthyroid at thirty months after withdrawal of the drug.

Most of the recurrences of hyperthyroidism were noted within the first few months after withdrawal of the drug. However, a few of the patients had the onset of their recurrences up to six years after cessation of therapy.

The data were analyzed to determine what factors had prognostic significance regarding the success of a course of therapy with antithyroid drugs. The following factors were found to be of no significance: age, sex, race, initial size of goiter, duration of therapy, and rapidity of response to therapy. In contrast, a decrease in the size of the gland during the course of therapy or the presence of a small gland at the end of therapy (1½ times normal size or less) were found to increase the likelihood of a long-term remission.

SURGERY

SAMUEL P. ASPER, JR.

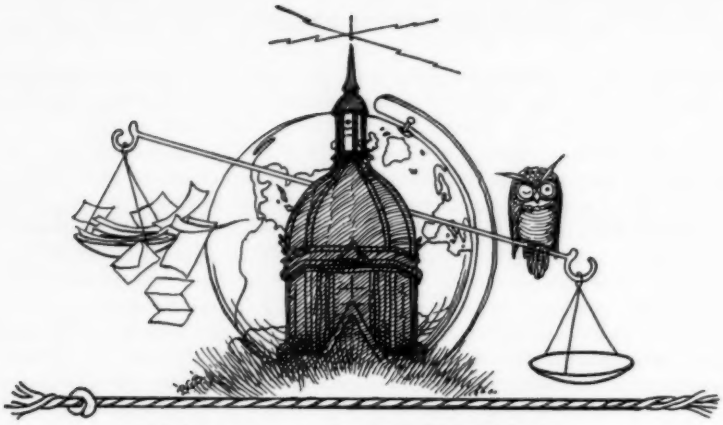
During the twelve year period 1946 through 1957 three hundred and seven operations for toxic goiter were performed at The Johns Hopkins Hospital. In 1952 and 1953 the number of operations performed annually was 36 and 32 respectively. In subsequent years the number of operations done each year diminished to about 20 to 25. The number of patients treated with

radioiodine increased somewhat in recent years. The number of patients treated annually with ablative therapy (that is, either subtotal thyroidectomy or radioiodine) has remained at about 50.

Of the 307 patients, 19 were treated preoperatively with thiouracil and iodine, 212 with propylthiouracil and iodine, 20 with methimazole and iodine and one with neomercazole and iodine. Also 9 patients, most of whom were known to be sensitive to iodine, were given only propylthiouracil preoperatively. Thirty-nine received only iodine, as potassium iodide or Lugol's solution. Four had no preoperative antithyroid therapy. Of the remaining three patients, one was in remission following radioiodine therapy and two following long-term antithyroid drug treatment; in each a persistent nodular goiter was removed.

No patient died either during the operation or as a direct consequence of it. Only six serious complications resulted, an incidence of two per cent; there were four patients with permanent hypothyroidism and two with permanent bilateral vocal cord paralysis. In all, however, an additional 26 patients had transient hypoparathyroidism and nine unilateral vocal cord paralysis, some transient and some permanent. Also, seven patients had a recurrence of hyperthyroidism following operation and 12 developed myxedema. Hematoma of the wound occurred in 12 patients. The overall morbidity rate was 23.5 per cent.

The data show that surgery remains an effective and safe method of treatment of toxic goiter.



BOOK REVIEWS

Handbook of Respiration. Analysis and Compilation by PHILIP L. ALTMAN, JOHN F. GIBSON, JR. AND CHARLES C. WANG. Edited by DOROTHY S. DITTMER AND RUDOLPH M. GREBE. Prepared under the direction of the Committee on the Handbook of Biological Data, Division of Biology and Agriculture, The National Academy of Sciences, The National Research Council. 403 pp., $7.50. W. B. Saunders Co., Philadelphia, Pa.

This book contains a vast quantity of data in graphic and tabular form covering animals from protozoa to man and plants from fungi to trees. It begins with an excellent section on basic physical and chemical data and continues with sections on basic respiratory anatomy, lung volumes and pulmonary function, respiratory characteristics of blood, erythrocyte and blood pigments, pulmonary mechanics, effects of exercise and variations in inspired P_{O_2}, respiratory control, effects of drugs, and metabolic rates of intact organisms and of isolated tissues in both plants and animals. Appendices include formulae for estimating body surface area, standard symbols, respiratory equations, and a summary of values useful in pulmonary physiology.

Data are presented clearly and complete references are given.

This rich source of information would be a valuable addition to general libraries and to the libraries of departments of physiology, laboratories concerned with ventilation, blood flow, and gas exchange and workers in any of these fields.

RICHARD H. SHEPARD

Therapeutic Exercise, Vol. III of Physical Medicine Library. Edited by SIDNEY LICHT. 893 pp., $16.00. Elizabeth Licht, Publisher, New Haven, Conn.

Exercise is the most important therapeutic tool in rehabilitation. It is important in preventing contractures, in maintaining muscle tone and coordination while spontaneous recovery takes place, and in developing new patterns to overcome deficiencies which may be lost permanently. To be effective, exercise must be applied to the right muscles at the proper time in suitable amounts for a definite time period in an appropriate form. The purpose of this book is to answer any question which may arise in the use of therapeutic exercise. Thirty-six authorities have contributed to this book.

The first three (of a total of thirty-five) chapters are brief summaries of the physiology and mechanics of muscular activity. These are followed by five chapters on functional anat-

omy and methods of measuring muscle strength and joint range. Subsequent chapters describe techniques of therapeutic exercise, including descriptions of a therapeutic gymnasium, proprioceptive facilitation and crutch and cane exercises, in addition to the more commonly used methods. There is a good chapter on the history of therapeutic exercise.

The second half of the book is concerned with the application of therapeutic exercises to different diseases. In addition, to the common orthopedic and neurological diseases, there are excellent chapters on the use of exercise in mental diseases, heart disease, obstetrics, vascular disease, ophthalmology and in the healthy person.

The editor has made a major contribution to the field of therapeutic exercises by seeing the need for such a book and by cajoling such a distinguished group of experts into contributing to it. Therapeutic exercise has wide applications in nearly all fields of medical practice, so that the book should be of interest to all physicians.

DOUGLAS CARROLL

Surgery and Surgical Nursing, 3rd edition. By EDWARD S. STAFFORD AND DORIS DILLER. 469 pp., $5.00. W. B. Saunders Co., Philadelphia, Pa.

Not only has the title of the previous edition *A Textbook of Surgery for Nurses*, been changed, but the new edition is an extensive revision of the previous one published in 1954. The basic arrangement of the volume is considerably altered with a new index which is a great deal more concise.

Interest in this precisely organized and well written book should include all those concerned with the art and science of surgical nursing. The book is designed for the student nurse and is intended to expose her to this highly complex specialty. Undoubtedly this volume will be of considerable value as a guide for those concerned with nursing education. The broad spectrum of surgery and its allied subspecialities are covered quite thoroughly, each of the subspeciality sections being written by an authority in the field.

The illustrations are good and in many instances new to this edition. Actual photographs have been substituted for some drawings and are an improvement. A successful attempt has been made to include in this edition many of the present advances in surgery.

The book is highly recommended for student nurses and all those concerned with the teaching of surgical nursing.

WILLIAM F. RIENHOFF, III

Biophysical Principles of Electrocardiography, Vol. 1. By ROBERT H. BAYLEY. 237 pp., $8.00. Paul B. Hoeber, Inc., New York, N. Y.

Here the clinical electrocardiographer is given a clear and consistent interpretation of the various details of the ECG, in terms of the Bayley-Wilson theory of the heart's field. This approach is based on simplified postulates about cardiac depolarization, body shape and conductivity. Using these, one can clearly picture how infarction and injury to the myocardium might account quantitatively and in detail for the many characteristic features of the normal and pathologic ECG. The chapters on T wave and injury potential are specially helpful.

Unfortunately, the Wilson model does not accept the physical facts now known of depolarization, of body contour referred to heart, or of tissue conductivity. Consequently the "biophysics" here presented, differs so much from actuality that this analysis should be regarded more as an aid to memory, than an adequate account for the existing ECG current-field. The "unbounded conductor" approximation (used throughout) is up to 300 per cent off; the conductive homogeneity is up to 100 per cent off; the depolarization pattern postulated is significantly erroneous. The extensive work of Burger, Schmitt, Schwann, Helm,

Frank, Scher and Gabor, is mainly neglected. These have shown that the Wilson-Helmholz model though free of commitment to the dipole concept, is too restrictive and cumbersome to use as a biophysical basis.

If the title were not "Biophysics" these criticisms would be pointless. But in his physical approach, the author has also neglected dimensional analysis, the fact that the heart is a current rather than a voltage generator, and the multipole nature of the near field. "Intrinsicoid" chest patterns are still stressed, though now unacceptable theoretically and practically. The gradient vector sG, being the sum of non-simultaneous components, can have no physical existence; indeed the clinical usefulness of this construct is not widely accepted. The QRS and T "loops", cancellation effects and composite leads are not even mentioned, though they are now central to our biophysical understanding of the ECG and its quantitative interpretation.

Were the book entitled "Wilson Interpretation of the ECG", the prospective reader could use to advantage the intelligible and consistent model of ECG etiology here presented, without expecting that he find the actual physics now known to be involved. However, the last fifteen years have brought out physical factors which make the interpretation of Wilson inadequate to account for present scientific observations. The book is therefore most useful to the clinician who wants a clear, simple explanation of what he sees, but does not expect help in following current biophysical research in electrocardiography.

Technically, Dr. Bayley's volume is beautifully executed, and profusely illustrated with explanatory drawings and electrocardiograms. The mathematical appendix, in this case highly exacting, is accurate and clear. The index is well suited to the readers it aims to serve.

SAMUEL A. TALBOT

Safety Techniques for Radioactive Tracers. By J. C. BOURSNELL. 68 pp., $1.75. Cambridge University Press, New York, N. Y.

This little book should be read by all beginners before entering into the use of radioactive tracers, because the safety techniques set forth are clearly the result of experience by people who have perceived the radioactive hazards "on the job". Positive solutions have been prescribed for the hazards presented.

Such American readers will find that preliminary study of Dr. Boursnell's book will provide them with sturdy pegs upon which to hang the knowledge they need from the more formidable National Bureau of Standards handbooks.

A bibliography at the end of the book provides a guided reading list of selected books for even the most experienced radioisotope user.

R. P.

Roots of Modern Psychiatry. Essays in the History of Psychiatry. By MARK D. ALTSCHULE. 184 pp., $5.75. Grune & Stratton, New York, N. Y.

If Dr. Altschule set out to show that the dictum of "nothing new under the sun" holds good for psychiatry too, he has certainly succeeded. He demonstrates that anxiety was given due consideration long before our century and that speculation on the seat of the soul and its problematic parts was rife before and after Galen. Griesinger is quoted at length to document the systematic discussion of the Ego around 1850, and the next chapter deals with "the growth of the Concept of Unconscious Cerebration before 1890". The allegorical interpretation of symptoms by psychosomatic medicine is traced back to antiquity, and so is the preponderant role assigned to sexual etiology. A large number of excerpts attest the widespread accusation of progressing civilization as a causative factor of mental disease, and kindness, shock therapy, suggestion, electro-therapy, were among the many eclectic ways of finding a

cure for it. While most chapters show an overt or implied criticism of Freud and his school, this criticism becomes the main topic of the last chapter. A return to the intuitive and subjective methods of some Greek philosophers (Plato!) together with a misuse of Greek mythology, and bad scholarship into the bargain, are imputed to the Freudians.

Dr. Altschule has read widely, and his book is stimulating. It is an excellent antidote to dogmatism. Nobody will fail to find something of interest among his aperçus and the many quotations he gives. The book is not a history of psychiatry, nor did Dr. Altschule intend it as such. However, his method of presenting quotations largely out of context and without a developmental chain gives the book a somewhat aphoristic character. The reader is not sure that the parallels with modern ideas always go very deep. For instance, it seems fair to neither Galen nor Freud to state that "the modern Id, Ego, and Super-Ego might be regarded as Galen's three souls brought up to date" (p. 50).

Having learned that practically everything was thought or done before, with doubtful results, the reader wonders what is left for the psychiatrist to place his confidence in. If I understand Dr. Altschule rightly, his advice to the clinician is to wait "for modern physiology and experimental psychology to provide the data for a valid theory of behavior."

OWSEI TEMKIN

BOOKS RECEIVED FOR REVIEW

British Medical Bulletin. Vol. 14, No. 3, Sept. 1958. Metabolism of Lipids. 278 pp., $3.25. Published by the Medical Department, The British Council, London, England.

Ciba Foundation Colloquia on Ageing, Vol. 4. Water and Electrolyte Metabolism in Relation to Age and Sex. Edited by G. E. W. WOLSTENHOLME AND CECILIA M. O'CONNOR. 327 pp., $8.50. Little, Brown & Co., Boston, Mass.

Ciba Foundation Symposium. Neurological Basis of Behaviour. Edited by G. E. W. WOLSTENHOLME AND CECILIA M. O'CONNOR. 400 pp., $9.00. Little, Brown & Co., Boston, Mass.

Deficiency Disease. By RICHARD H. FOLLIS, JR. 577 pp., $14.75. Charles C Thomas, Springfield, Ill.

Diagnostic Bacteriology, 5th edition. By ISABELLE G. SCHAUB, M. KATHLEEN FOLEY, ELVYN G. SCOTT AND W. ROBERT BAILEY. 338 pp., $4.75. The C. V. Mosby Co., St. Louis, Mo.

Emergency War Surgery. U. S. Armed Forces Issue of Nato Handbook Prepared for Use by the Medical Services of Nato Nations. 411 pp., $2.25. United States Government Printing Office, Washington, D. C.

Pathophysiology in Surgery. By JAMES D. HARDY. 704 pp., $19.00. The Williams & Wilkins Co., Baltimore, Md.

Physiological Bases of Psychiatry. American Lecture Series. Compiled and Edited by W. HORSLEY GANTT. 344 pp., $10.50. Charles C Thomas, Springfield, Ill.

Poisoning. A Guide to Clinical Diagnosis and Treatment, 2nd edition. By W. F. VON OETTINGEN. 627 pp., $12.50. W. B. Saunders Co., Philadelphia, Pa.

Rehabilitation Medicine. By HOWARD A. RUSK. 527 pp., $12.00. The C. V. Mosby Co., St. Louis, Mo.

Schizophrenia. By MANFRED SAKEL. 335 pp., $5.00. Philosophical Library, New York, N. Y.

ACQUIRED TOLERANCE TO BACTERIAL ENDOTOXIN: THE PYROGEN AUGMENTING EFFECT OF SERUM[1, 2]

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A characteristic febrile response develops in many animal species, including man, following the intravenous inoculation of Gram-negative bacterial endotoxins. The induced fever occurs following a lag period after injection of the toxin, is frequently biphasic, and persists for only a few hours. Repeated daily injection of endotoxin results in a decreasing febrile response often referred to as *pyrogen tolerance*. After about 7 to 14 days a maximum degree of tolerance is achieved to the daily doses of toxin given and decreasing tolerance can be detected for 2 to 3 weeks after the injections are discontinued.

The biphasic fever response observed on the first day of endotoxin injection usually is not present with the second or third daily inoculation, and when maximum pyrogen tolerance is obtained, the lag-period is prolonged and the degree of fever is reduced. Tolerance is frequently not complete as a small febrile response may continue to develop after toxin is given. Induction of tolerance to the pyrogenic action of one endotoxin results in the development of tolerance to endotoxins derived from heterologous Gram-negative bacteria (1–4). Although most bacterial endotoxins are good antigens there is no apparent relationship between detectable levels of serum antibody and the presence of pyrogen tolerance (1, 2, 3, 5, 6). This is further indicated by the usual inability to passively transfer tolerance with serum (1, 2). Repeated daily injection of endotoxin produces tolerance or resistance to other biological actions of the pyrogen, including its lethal (7, 8) and leucopenic effects (9, 10), and its ability to produce the Shwartzman phenomenon. (1, 11, 12, 13).

The febrile response of rabbits to endotoxin is greater when the pyrogen is mixed with normal serum for a short period of time prior to injection (14–19). This increase in pyrogenic effect of the toxin is characterized by shortening of the lag-period and a higher fever. The pyrogen augmenting effect of normal serum is readily demonstrable in tolerant animals, in which case tolerance seems to be overcome (14, 16). Serum from tolerant rabbits, furthermore, fails

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[2] National Cancer Institute, Bethesda, Maryland.

to enhance the febrile response to endotoxin (14, 16, 18). The inability of tolerant rabbit serum to augment pyrogen fever as normal serum does may be attributable to the development of serum "inhibitors." This report is presented to delineate the relationship between the influence of serum on endotoxin fever and acquired tolerance.

Materials and Methods

Bacterial Endotoxin: Purified endotoxins derived from *Shigella flexneri* (20) and *Serratia marcescens* (P-35)[2] were used. The endotoxins were dissolved in 0.85 per cent NaCl and were used in concentrations of 0.01 mcgm./ml. and 5 to 10 mcgm./ml., respectively.

Animals: Normal male rabbits of mixed breed and color, weighing 2 to 3 Kg. were used in the experiments.

Rabbit serum: The rabbits were bled by cardiac puncture using sterile precautions. Blood was collected in pyrogen free 50 ml. tubes, allowed to clot, centrifuged at 2500 r.p.m. for 30 minutes at 4° C. and the serum was collected and stored at 4° C.

Measurement of Temperature: The febrile response of rabbits was measured by an indwelling rectal resistance coil and a Telethermometer (21) every 30 minutes for 1 hour prior to intravenous injection of endotoxin and for 5 hours thereafter. Fever indices were measured by planimetry (1).

Tolerant rabbits: Normal rabbits were made tolerant to 0.01 mcgm. *Shigella flexneri* endotoxin or 5 to 10 mcgm. P-35 by daily intravenous inoculation for 2 to 3 weeks. Development of tolerance was confirmed by measurement of temperature responses on the first and last days of inoculation.

EXPERIMENTAL RESULTS

Appearance of tolerance and disappearance of the pyrogen augmenting effect of serum

Normal rabbit serum will enhance the febrile response of animals when mixed with endotoxin prior to injection, whereas tolerant rabbit serum will not (14, 16, 18). To study the relationship of the disappearance of this effect of serum upon the pyrogenic action of endotoxin and the development of pyrogen tolerance, the following experiments were done.

A group of 12 rabbits was made tolerant to 5 mcgm. P-35 by repeated daily injection for ten days, and temperatures were recorded on days 1, 3, and 7. The fever of individual animals corresponded closely to the mean for the group. On days 2, 4, and 8, prior to the daily injection of endotoxin, 10 ml. of blood was obtained from each rabbit and the corresponding sera were separated and pooled. A second group of 12 rabbits were rendered tolerant to 0.01 mcgm. of Shigella endotoxin. These animals were then given intravenous injections of 0.01 mcgm. Shigella endotoxin which had been incubated for 30 minutes at 37°C. with 4.0 ml. of the serum obtained from the rabbits given P-35 every day. Each serum sample was tested in 4 animals in which temperatures were measured.

The development of pyrogen tolerance to the P-35 toxin is shown in Figure 1, illustrating the disappearance of the biphasic temperature curve, reduc-

tion in fever, and lengthening of the lag-period. Figure 2 shows the pyrogen augmenting effects of serum obtained on day 1, 3, and 7 from rabbits becoming tolerant to P-35 when tested in animals tolerant to Shigella endotoxin. The Fever Indices of the mean temperature curves shown in Figures 1 and 2 are plotted in Figure 3, and a close correlation is seen between development of pyrogen tolerance and the disappearance of the pyrogen augmenting effect of serum.

Disappearance of tolerance and reappearance of pyrogen augmenting effect of serum

Animals tolerant to the pyrogenic action of bacterial endotoxin gradually lose their tolerance within about 3 weeks when daily injections of the toxin

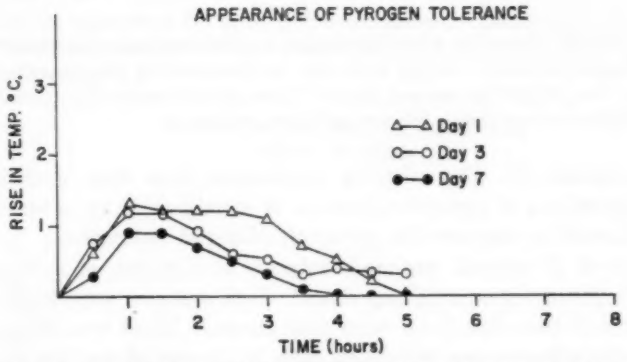

FIG. 1. Appearance of tolerance to the pyrogenic action of 5 mcgm. P-35 toxin given intravenously every day. Temperatures recorded on day 1, 3, and 7.

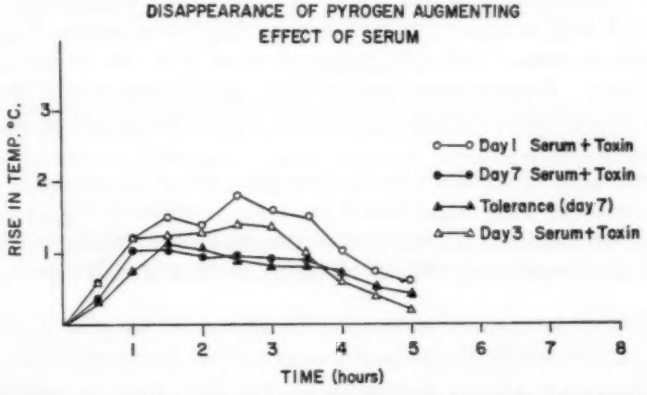
DISAPPEARANCE OF PYROGEN AUGMENTING EFFECT OF SERUM
RISE IN TEMP. °C.
TIME (hours)
Day 1 Serum + Toxin
Day 7 Serum + Toxin
Tolerance (day 7)
Day 3 Serum + Toxin

FIG. 2. Disappearance of the ability of rabbit serum, obtained after the first, third, and seventh daily injection of 5 mcgm. P-35, to augment the pyrogenic action of 0.01 mcgm. Shigella endotoxin in rabbits tolerant to Shigella endotoxin.

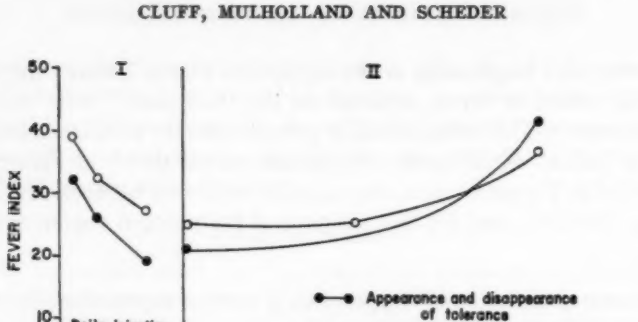

FIG. 3. Graphic illustration of the appearance and disappearance of tolerance of rabbits to the pyrogenic action of 5 mcgm. P-35, and the corresponding disappearance and reappearance of the pyrogen augmenting effect of these rabbits' serum when tested with 0.01 mcgm. Shigella toxin in animals tolerant to Shigella endotoxin.

are discontinued (1). The following experiments were done to determine if this disappearance of pyrogen tolerance is accompanied by a return of the ability of serum to augment the pyrogenic effects of endotoxin.

A group of 12 animals was made tolerant to 5 mcgm. of P-35 endotoxin as described in the preceding experiment. Temperatures were taken on days 1 and 10 and then injections were discontinued. Blood was obtained from 3 groups of 3 animals, respectively, on days 11, 24, and 38, and the corresponding sera were pooled. Eight of the 12 animals not bled on day 38 were tested for pyrogen tolerance with an intravenous injection of 5 mcgm. P-35. Each of the 3 serum specimens was tested for its pyrogen augmenting effect in groups of 4 rabbits tolerant to 0.01 mcgm. Shigella endotoxin. Four ml. of serum were incubated with 0.01 mcgm. toxin at 37°C. for 30 minutes prior to inoculation. Temperatures were recorded and compared with the febrile response of the tolerant animals given 0.01 mcgm. Shigella toxin in 4 ml. of 0.85 per cent NaCl.

Disappearance of tolerance to the pyrogenic action of endotoxin 28 days *after discontinuing* daily injections of the toxin is shown in Figure 4. As the the animal's tolerance to the toxin is lost, there is a corresponding reappearance of the pyrogen augmenting effect of his serum, as illustrated in Figures 3 and 5.

Abolishment of pyrogen tolerance with Thorotrast, and the effect of Thorotrast on the ability of a tolerant animal's serum to augment fever

Intravenous injection of Thorotrast abolishes a rabbit's tolerance to the pyrogenic action of endotoxin (11, 22, 23, 24). To determine if this effect of

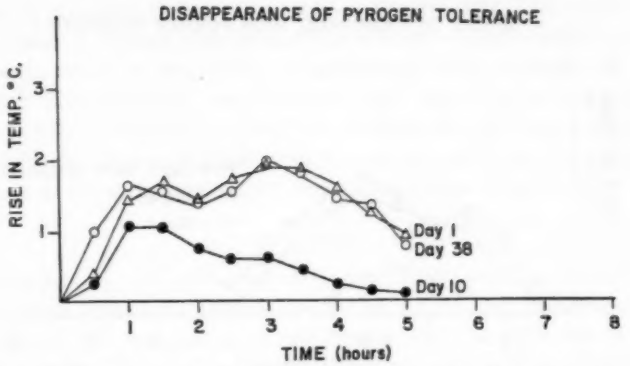

FIG. 4. Febrile response on day 1, 10, and 38 of a group of rabbits given 5 mcgm. P-35 daily for 10 days then rested for 28 days; demonstrating the appearance and disappearance of pyrogen tolerance.

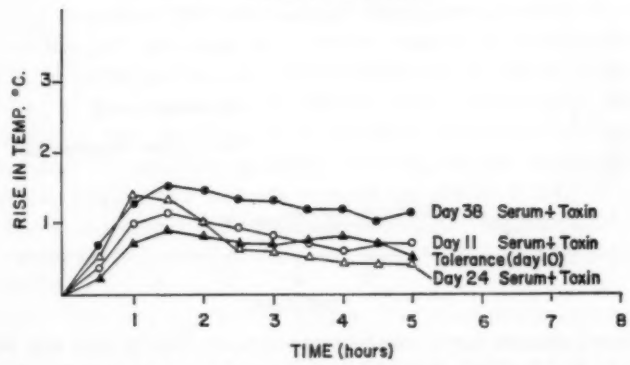

FIG. 5. Reappearance of the ability of rabbit serum, obtained on the first, fourteenth, and twenty-eight day *after* development of pyrogen tolerance to 5 mcgm. P-35, to augment the pyrogenic action of 0.01 mcgm. Shigella endotoxin in rabbits tolerant to the Shigella toxin.

Thorotrast in tolerant animals is accompanied by a reappearance of the pyrogen augmenting properties of serum the following experiment was performed.

A group of 12 rabbits was rendered tolerant to 5 mcgm. of P-35. Six of the rabbits were given 9 ml. of Thorotrast intravenously the day after the last daily injection of toxin. Three of the Thorotrast treated animals and all of the other rabbits were exsanguinated by cardiac puncture 2 days after the

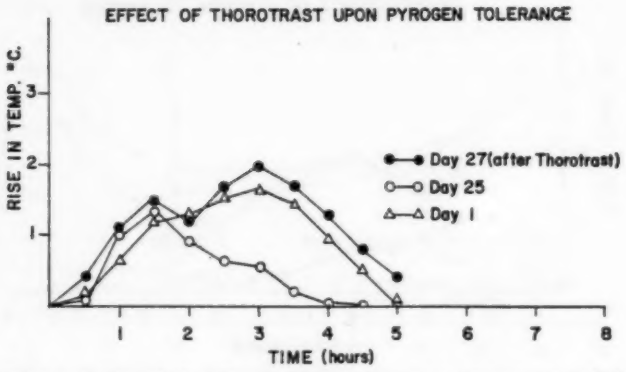

FIG. 6. Demonstration of the disappearance of pyrogen tolerance in rabbits given 5 mcgm. P-35 daily for 25 days, after intravenous injection of 9 ml. Thorotrast.

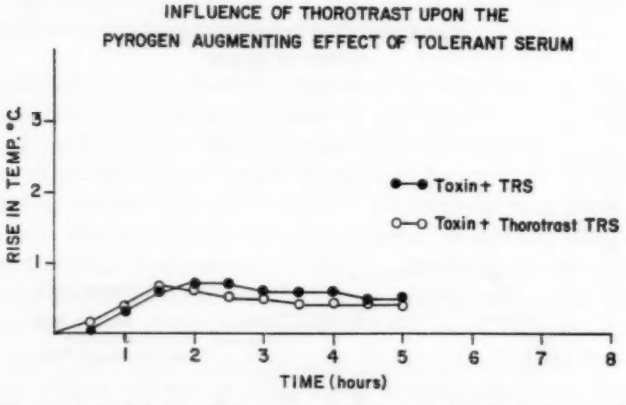
INFLUENCE OF THOROTRAST UPON THE
PYROGEN AUGMENTING EFFECT OF TOLERANT SERUM
RISE IN TEMP. °C.
TIME (hours)
Toxin + TRS
Toxin + Thorotrast TRS

FIG. 7. Serum obtained from rabbits tolerant to 5 mcgm. P-35 18 hours after intravenous injection of 9.0 ml. Thorotrast, tested for its ability to enhance the pyrogenic action of 0.01 mcgm. Shigella toxin in rabbits tolerant to the pyrogenic action of Shigella toxin.

last injection of toxin and the serum was collected and pooled as before. The remaining 3 animals were given 5 mcgm. of P-35 intravenously and their febrile responses were recorded and compared with the temperature curves of the tolerant animals prior to injection of Thorotrast. Another group of rabbits tolerant to 0.01 mcgm. Shigella endotoxin was used to test the pyrogen augmenting effect of serum from the tolerant rabbits given Thorotrast 18 to 24 hours previously and from the tolerant animals not given Thorotrast. Four ml. of the respective sera were mixed with 0.01 mcgm. Shigella endotoxin and these were incubated for 30 minutes at 37°C. prior to injection. Temperatures were recorded as described above.

The loss of pyrogen tolerance by rabbits treated with Thorotrast is illustrated in Figure 6. Serum withdrawn from tolerant animals failed to enhance the pyrogenic action of endotoxin, as anticipated, and treatment of tolerant rabbits with Thorotrast did not influence their serum in its effect on endotoxin fever (Fig. 7). Serum obtained from normal rabbits treated with Thorotrast, in the same way as in these experiments, enhanced the pyrogenic action of endotoxin as did sera from normal rabbits not given Thorotrast.

DISCUSSION

Augmentation of the pyrogenic action of endotoxin by normal rabbit serum has been repeatedly demonstrated (14, 16, 18, 19) and augmentation by whole blood (17) and leucocyte rich plasma (15) has been shown to occur in human beings. This effect occurs after serum and endotoxin have been in contact for a very short period of time at 37°C. or 4°C. (16, 18, 19, 21, 25). Prolonged contact of endotoxin with normal rabbit serum at 37°C., however, results in reduction of the toxin's pyrogenic action, probably attributable to the properdin system (14). The pyrogen augmenting property of rabbit serum is apparently not associated with combination of endotoxin to a protein constituent of the serum (26), but the exact mechanism by which this phenomenon occurs has not been defined. It is not related to a non-specific effect attributable to serum protein, as tolerant rabbit serum fails to augment fever although its protein content is little different than normal rabbit serum (27).

The possibility that development of pyrogen tolerance is associated with appearance of a non-antibody inhibitor, however, has not been clarified. The stoichiometric relationship between volume of serum added to endotoxin and the degree of pyrogen augmentation, as well as the observation that the reaction occurs at 4°C. certainly does not indicate that an enzymatic process is involved (14).

The studies reported here have shown a close relationship between the development and disappearance of pyrogen tolerance and the disappearance and reappearance of the pyrogen augmenting effect of the rabbit's serum. This suggests that the property of serum which enhances the febrile response may be important in the pathogenesis of endotoxin fever and in the mechanism of pyrogen tolerance. However, the observation made here that treatment of the rabbit with Thorotrast results in a loss of pyrogen tolerance unassociated with a reappearance of the pyrogen augmenting effect of serum indicates that other phenomena are also involved in the development of tolerance and in the pathogenesis of fever.

Tolerance to the pyrogenic action of endotoxin is associated with a change from normal in the distribution of the toxin following intravenous inoculation, and is characterized by a more rapid clearing of the toxin from the blood (11, 28, 29). Persistence of this change in distribution of injected endotoxin after

tolerance has subsided is probably attributable to the presence of serum antibody (28). This indicates that change in distribution alone is not the sole mechanism of pyrogen tolerance but may be contributory.

Leucopenia is less pronounced following injection of endotoxin into the pyrogen tolerant animal, and this is associated with a disappearance of the inhibitory influence of endotoxin upon the migration of leucocytes (30). In addition, endotoxin is taken up more avidly by circulating leucocytes in tolerant than in normal rabbits (28).

As illustrated in this study, as well as before (11, 22, 23, 24), "blockade" of the reticulo-endothelial system (Thorotrast) can overcome pyrogen tolerance, and this suggests a fourth possible mechanism in tolerance to endotoxin fever. There is little evidence which relates the activity of the adrenal cortex to pyrogen tolerance (31), and there is nothing to suggest that the thermoregulatory centers of the brain become refractory to the pyrogenic action of endotoxin (31).

It would appear, therefore, that at least 4 mechanisms may be operative in the development of tolerance to the febrile effect of endotoxin: (1) the pyrogen augmenting effect of serum-demonstrated by the close relationship between the influence of serum on the pyrogenic action of the toxin and development of tolerance, (2) blood leucocytes—demonstrated by the resistance of leucocytes from pyrogen tolerant rabbits to the inhibiting influence of endotoxin upon leucocyte migration, (3) reticulo-endothelial (R-E) system—demonstrated by the ability of R-E blockade to abolish pyrogen tolerance, and (4) serum antibody—demonstrated by the influence of serum antibody upon the distribution of endotoxin following intravenous injection.

A number of effects of serum upon the biological action of endotoxin has been described and are listed in Table I. The relationship of these observations to the pyrogen augmenting effect of normal serum, is far from clear. Many of the studies performed have not included close attention to the fact that augmentation of fever is demonstrable only with minimum pyrogenic doses of endotoxin and that enhancement of fever by serum is most readily demonstrated in pyrogen tolerant animals. In addition, some of the studies have not defined the relationship between the observed effects of serum and the properdin system (32, 33, 34). Furthermore, the importance of period of contact and temperature at which serum and endotoxin are mixed in determining the observed effects indicate that more than one action of serum upon endotoxin is probable. In the studies reported here, however, we have been concerned only with effects of serum upon the pyrogenic action of endotoxin after the two have been mixed for a short period of time prior to inoculation, and this may have no relationship to the effects of serum upon the lethal action, hemagglutinin inhibition, or alteration of physical-chemical or immunological properties of endotoxin observed when serum and toxin are mixed for a longer period of time at 37° C.

TABLE I

Effects of Serum on the Biological Action of Endotoxin

Author	Observed Effect	Source of Serum	Comments
1. Farr and LeQuire (16), Cluff (21), Cluff and Bennett (14), Gerbrandy *et al.* (17)	Augmentation of pyrogenic action	Rabbit (serum), human (whole blood)	Not temperature dependent, stoichiometric, serum property heat stable
2. Hegeman (32, 33), Cluff and Bennett (14)	Inhibition of pyrogenic action	Human and rabbit	Temperature dependent, serum property heat labile, related to properdin system
3. Farr and LeQuire (16)	Inhibition of pyrogenic action	Rabbit (pyrogen tolerant)	
4. Ho and Kass (38)	Inhibition of lethal action of crude endotoxin in mice	Human, rabbit, and rat	Heat stable, not attributable to properdin system
5. Kelly *et al.* (40)	Inhibition of lethal action in mice	Rabbit	
6. Landy (39)	Inhibition of production of hemorrhagic necrosis of mouse sarcoma	Human	Heat labile, temperature dependent (37°C. for 4 hours), probably not attributable to properdin system
7. Stauch and Johnson (41)	Alteration in immunologic properties	Rabbit	Heat stable, temperature dependent (37°C. for 4 hours)
8. Neter *et al.* (42)	Inhibition of adsorption to erythrocytes	Human	Possibly attributable to cholesterol or lecithin
9. Cluff (21)	Change in diffusion properties in gel	Rabbit	
10. Pillemer and Landy (39)	Change in turbidity, dispersion and sedimentation	Human	
11. Petersdorf and Bennett (36), Mulholland and Cluff (43)	Renders pyrogenic action of endotoxin heat labile	Dog and rabbit	
12. Rowley (44)	Splits off phosphorus	Human, rat, and mice	Probably related to properdin system

The "endogenous pyrogen" described by Atkins and Wood (35) which appears in the blood of rabbits after intravenous injection of endotoxin has properties dissimilar from the inoculated toxin. "Endogenous pyrogen" is heat labile, produces fever in rabbits tolerant to the pyrogenic action of endotoxin, will not induce tolerance in normal animals and may be a product of leucocytes (28). There are certain similarities between "endogenous pyrogen" and endotoxin mixed with normal serum, however, which have not enabled a complete differentiation. Endotoxin in serum is heat labile (36), will not induce tolerance to the augmented febrile response and produces fever in rabbits tolerant

to the pyrogenic action of endotoxin (14). In addition, "endogenous pyrogen" does not appear following injection of endotoxin into pyrogen tolerant animals, and endotoxin mixed with tolerant rabbit serum will not enhance the pyrogenic action of endotoxin in normal or tolerant rabbits (16, 37). Treatment of the pyrogen tolerant animal with Thorotrast, however, is associated with a reappearance of "endogenous pyrogen" in the blood following endotoxin injection; whereas, in the studies reported here, this treatment is not associated with a restoration of the pyrogen augmenting property of the animal's serum. This observation suggests that "endogenous pyrogen" and the altered pyrogen produced by mixing of endotoxin with normal serum are indeed different substances.

SUMMARY

The gradual development of tolerance towards the pyrogenic action of endotoxin, induced by repeated daily injection of the toxin into rabbits, is closely associated with a gradual disappearance of the ability of the animal's serum to augment the febrile response to endotoxin. Similarly, disappearance of the induced tolerance over a 3 week period, after discontinuing the repeated daily injections of toxin, is related to a return of the ability of the animal's serum to augment endotoxin fever. This relationship between acquired tolerance to bacterial pyrogen and the influence of the animal's serum upon the endotoxin's pyrogenic action suggests that the pyrogen augmenting effect of serum may be important in the mechanism of tolerance as well as in the pathogenesis of fever.

Treatment of the tolerant rabbit with Thorotrast abolishes pyrogen tolerance but is not associated with a reappearance of the pyrogen augmenting effect of the animal's serum. This indicates that other mechanisms, including the reticulo-endothelial system, are also operative in development of tolerance to the pyrogenic action of endotoxin.

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EXPERIMENTAL THROMBOCYTOPENIA AND PLATELET AGGLUTINATION[1]

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The agglutination of antigenic particles has long been utilized by immunologists as a means of demonstrating that a particulate antigen has reacted with an antibody. In recent years investigators in the field of immunohematology have employed the agglutination of platelets as an end point in the attempt to demonstrate the presence of anti-platelet factors in the serum of some patients with thrombocytopenia. This approach has been utilized in the search for anti-platelet substances, both naturally occurring and acquired.

The presence of an anti-platelet factor in the plasma of some patients with thrombocytopenia was convincingly demonstrated by Harrington and his associates (1). In their experiments, transfusion of plasma from thrombocytopenic patients caused thrombocytopenia in the normal recipients. When platelet suspensions were mixed *in vitro* with serum from some patients with thrombocytopenic purpura, agglutination of the platelets was reported to occur (2). Numerous investigators have used this *in vitro* method to demonstrate platelet agglutinins.

In this laboratory attempts have been made to demonstrate platelet antibodies in the serum of a large group of patients with thrombocytopenic purpura (3). A variety of methods has been employed. Mixtures of the patient's serum with platelet suspensions prepared in several ways have been observed for clumping of platelets after varying periods and conditions of incubation. Serum samples from normal persons and from non-thrombocytopenic patients were used as controls. A certain amount of platelet clumping was often seen. However, this phenomenon was not very reproducible and at times occurred in the control serum as well as in that obtained from thrombocytopenic patients. There appeared to be little difference between the patients' serum and that of the controls in frequency and degree of platelet clumping.

[1] This investigation was carried out under Contract AT(30-1)1208 between the Atomic Energy Commission and The Johns Hopkins University, and was supported in part by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health.

[2] Part of this work was performed during the tenure of a Research Fellowship of The American College of Physicians—1956–1957. Present address: 1111 University Drive, Menlo Park, California.

In a further study of this problem (4) serum from a large number of individuals was examined for platelet agglutinins in such a way that the investigator was unaware of the source of the serum at the time of the test. Undiluted serum from patients with "idiopathic" thrombocytopenic purpura more often agglutinated platelets than did serum from patients with other diseases. However, serum from 5 per cent of a group of 105 normal persons who had never been thrombocytopenic nor immunized to platelets reproducibly clumped test platelets from a panel of donors. This platelet agglutinin appeared not to be related to the ABO system. Serum from a higher percentage of patients with acute and chronic diseases but with normal platelet counts was observed to agglutinate normal platelets. Clumping of platelets has been noted in the serum of some patients with cirrhosis of the liver, rheumatoid arthritis, steatorrhea, systemic lupus erythematosus and infectious mononucleosis. No difference could be demonstrated between the agglutinins encountered in the serum of thrombocytopenic patients and that found in the controls. The titer of the agglutinins was low, never exceeding 1:8.

Platelets have an inherent adhesive quality which becomes more pronounced during the process of blood coagulation. The end point of agglutination is therefore extremely vulnerable to flaws in technique which permit the initiation of coagulation. Furthermore, the occurrence of fibrinolysis in clotted blood was observed to be associated with the development of an extraordinary capacity of serum to agglutinate normal platelets (4).

Many of the descriptions of methods for identifying antiplatelet factors by platelet agglutination techniques have not emphasized the non-specific occurrence of platelet clumping. Adequate emphasis has not been given to the impossibility of distinguishing non-specific agglutination from that induced by specific anti-platelet substances. In the present study a specific platelet antibody was produced experimentally so that its properties could be studied. The experimental antibody can be used for the study of methods which may be suitable for the precise recognition of platelet antibodies in human subjects.

The work to be described includes observations on (1) a method for preparing an anti-platelet serum, (2) the antigenic qualities of the platelet when used for the stimulation of antibody formation and when employed as a particulate antigen for the detection of antibody, (3) the characteristics of platelet agglutination when a known, specific antibody is employed, and (4) some characteristics of the antiserum when tested *in vivo*.

Methods and Materials

Blood specimens for platelet preparations were collected through 18 gauge needles that had not been treated with a nonwetting agent. Syringes were treated with silicone.[3] All glassware which was used in the tests for platelet agglutination was treated with silicone which had been diluted tenfold with petroleum ether.

[3] Dri-Film SC87; General Electric Co.

Hematologic studies

The hematocrits were determined by the technique of Wintrobe. Platelets were counted by the phase contrast technique of Brecher and Cronkite in which 1 per cent ammonium oxalate is used as the diluent. The normal range in dogs is approximately 200,000 to 600,000 per cu. mm.

Technique for immunization

Albino rabbits weighing approximately 2,500 grams were immunized with platelets collected from mongrel dogs. The immunization was accomplished over a four week period. Injections of the antigen were made on four consecutive days of four consecutive weeks for a total of 16 injections. Blood for platelet preparations was collected from the jugular vein or femoral artery of the dog, and transferred to silicone treated tubes containing $\frac{1}{10}$ volume of 1 per cent disodium sequestrene. The platelets obtained from 10 ml. of blood were used for one injection into one rabbit. No adjuvant substance was used. Suspensions of platelet rich plasma were prepared by centrifugation at slow speed without regulation of temperature. (In an International Centrifuge, Size 1, Type SB, centrifugation at 1,000 r.p.m. for 15 minutes was satisfactory.) The platelet rich plasma was centrifuged for an additional 10 minutes at slow speed to provide a more complete separation of erythrocytes. A platelet button was obtained by centrifugation of the platelet rich plasma at high speed, and the supernatant plasma was discarded. To remove traces of residual plasma, the platelets were washed three times with isotonic saline solution. The final platelet suspension was made in saline at a concentration of about 1,200,000 platelets per cu. mm. One ml. of this suspension was injected into the marginal ear vein of the rabbit. The platelet suspensions were stored at 5°C. for not longer than 24 hours.

Using aseptic technique, blood was collected from the rabbit by cardiac puncture 3 to 5 days after the last injection of the antigen. The blood was allowed to clot at 37°C. for 12 hours. The serum was separated, and heated at 56°C. for 30 minutes. A platelet agglutination test was performed with the serum obtained from each rabbit to determine the titer of the antibody. An agglutinin titer of at least 1:40 was considered satisfactory. The acceptable sera were pooled; the antibody to the heterologous erythrocyte was absorbed, and the serum stored in small aliquots at −20°C.

Absorption of the anti-red cell factor

Because of the difficulty in separating completely erythrocytes from the platelet suspensions used for immunizing, the prepared antiserum contained an anti-erythrocyte factor as well as the antibody against platelets. Absorption of the red cell antibody was accomplished in such a manner as to avoid bacterial contamination. Red cells used for absorption were collected from the femoral artery of the dog into a Fenwal plastic bag containing ACD solution. The platelet rich plasma was separated by slow centrifugation and discarded. The red cells were resuspended in a volume of isotonic saline equal to the volume of platelet rich plasma removed. Very few platelets remained after three such separations.

The serum for absorption was inactivated at 56°C. for 30 minutes. The absorption was carried out at 5°C. with the dog erythrocytes constituting a 20 per cent suspension in the antiserum, and was continued until the antiserum no longer agglutinated a 2 per cent saline suspension of the dog erythrocytes.

Tests for platelet agglutination

Platelet rich plasma was prepared as previously described, using $\frac{1}{10}$ volume of 1 per cent disodium sequestrene or $\frac{1}{10}$ volume of ACD solution as the anticoagulant. Serum dilutions

were made in parallel using isotonic saline as the diluent. The reaction mixture, consisting of 0.5 ml. of platelet rich plasma and 0.5 ml. of serum was incubated at the desired temperature for varying time intervals. Agglutination was looked for both macroscopically and microscopically. The microscopic observations were made with the low power objective of the microscope with the reaction mixture in a depressed cell slide.

Experimental Results

in vitro observations

1. Naturally occurring, heterologous platelet agglutinins

When dog platelets, suspended in native plasma were incubated with normal rabbit serum which had been heated at 56°C. for 30 minutes, there was rapid agglutination of the platelets. This was not clearly visible on macroscopic examination but was evident microscopically. This phenomenon was not impressive and was not seen in serum dilutions greater than 1:2. The agglutination was the same when ACD solution or sequestrene was employed as the anticoagulant and occurred in similar degree at incubation temperatures of 5°, 25°, and 37°C.

2. Agglutination of platelets by an anti-platelet serum

When the rabbit anti-dog platelet serum was incubated with a platelet suspension from a dog, there was rapid, unequivocal agglutination of the platelets, with few free and isolated platelets remaining. Large conglomerates of platelets were formed. The reaction appeared to be complete within one hour, and proceeded equally well at incubation temperatures of 5°, 25°, or 37°C. The platelets lost much of their normal morphology when viewed under phase microscopy. The reaction was in no way fastidious. Temperature and platelet concentration were not critical factors. A platelet concentration in the platelet rich plasma of 200,000 per cu. mm. was satisfactory. When sequestrene was used as the anticoagulant, lysis of the platelets was not observed. However, when ACD solution was used in the preparation of the platelet rich plasma, there was lysis of the platelets in the reaction mixtures out to serum dilutions of 1/64, and lysis plus agglutination in the more dilute serum. The agglutination titer in both the ACD and sequestrene preparations was noted to be 1/256.

The large conglomerates of platelets were easily visible macroscopically within 30 minutes after the reactants were mixed. The agglutination in the higher dilutions of serum was unequivocal when viewed microscopically. The end point of agglutination was rapidly obtained, easily observed, entirely reproducible and clearly unequivocal and in sharp contrast to the controls where the platelets remained evenly suspended. This antiserum was capable of agglutinating the platelets from all mongrel dogs tested and its effect was in no way limited to the platelets of dogs used as donors for purposes of immuniza-

tion. The absorption of the serum with canine erythrocytes did not diminish the titer of the anti-platelet factor.

3. Naturally occurring heterologous erythrocyte agglutinins

The incubation of 2 per cent, platelet free, saline suspension of canine red cells with normal, heat inactivated, rabbit serum produced rapid agglutination of the red cells. The titer of this heterologous agglutinin was about 1:8. No hemolysis was observed.

4. Agglutination of heterologous erythrocytes by the rabbit antiserum

When 0.2 ml. of a 2 per cent, platelet free, saline suspension of canine red cells was incubated with 0.2 ml. of the unabsorbed rabbit-anti-dog platelet serum, there was agglutination of the red cells in the serum to a dilution of 1/320. If the serum had not been inactivated at 56°C., hemolysis was observed in the first few tubes.

Regardless of the care that had been taken to remove the erythrocytes from the platelet suspension used for immunization, it was extremely difficult to prepare a platelet rich plasma which was entirely free of erythrocytes. Thus, in immunizing the rabbit against platelets, an anti-red cell factor was obtained as well.

IN VIVO STUDIES

1. Results of the injection of normal rabbit serum and rabbit anti-dog platelet serum in the dog

Figure 1 depicts the response of a dog to an intravenous injection of 2 ml. of normal rabbit serum, a second injection of 6 ml. of normal rabbit serum, a third injection of 2 ml. of rabbit anti-dog platelet serum, and a fourth injection of 2 ml. of normal rabbit serum. All sera had been absorbed previously with dog erythrocytes. The first injection of 2 ml. of normal serum had virtually no effect on the platelet count, hematocrit, or white blood cell count. The injection of 6 ml. of normal rabbit serum on the 7th day, however, produced a very transient, but definite, decrease in the platelet count. There was no significant change in the hematocrit value or leucocyte count. The dog exhibited no systemic effects, and there was no evidence of intravascular hemolysis. On the 8th day an injection of 2 ml. of rabbit anti-dog platelet serum was given with the appearance of an immediate, severe thrombocytopenia. Within this period there was a transient leucopenia, but no significant change in the hematocrit. The dog exhibited no systemic reaction. There was no intra-vascular hemolysis. The thrombocytopenia persisted for 36 hours, and the platelet count returned to normal on the 5th day after the injection. On the 55th day, the same dog was given an injection of 2 ml. of normal rabbit serum. A severe reaction occurred within 15 minutes, with vomiting, defecation, and evidence of intra-

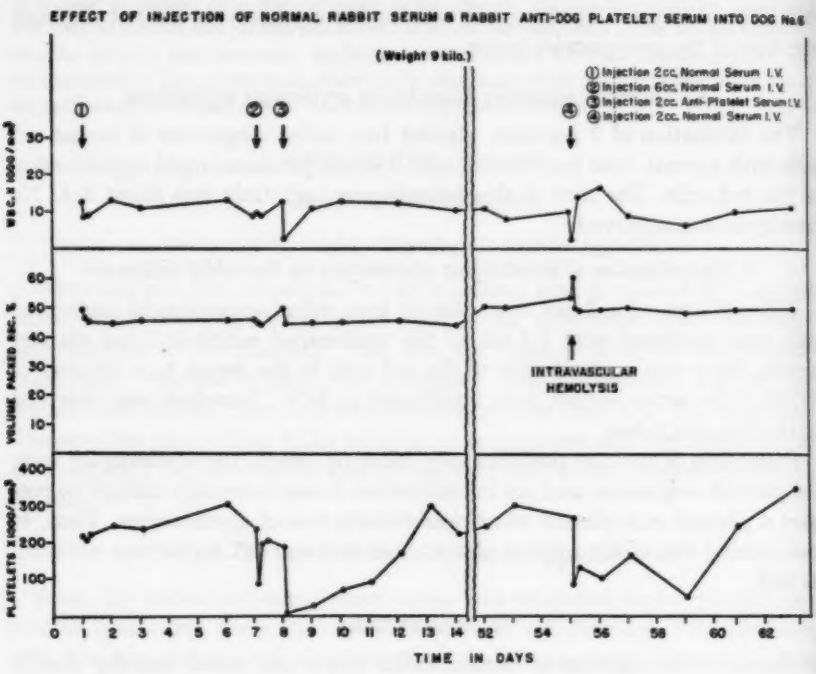
EFFECT OF INJECTION OF NORMAL RABBIT SERUM & RABBIT ANTI-DOG PLATELET SERUM INTO DOG No.5
(Weight 9 kilo.)
① Injection 2cc. Normal Serum I.V.
② Injection 6cc. Normal Serum I.V.
③ Injection 2cc. Anti-Platelet Serum I.V.
④ Injection 2cc. Normal Serum I.V.
INTRAVASCULAR HEMOLYSIS
TIME IN DAYS

FIG. 1

vascular hemolysis. There was moderate thrombocytopenia which persisted for several days. Initial leucopenia was followed by a moderate leucocytosis. At the time of the reaction there was a sharp rise in the hematocrit value. The pattern of this thrombocytopenic period in no way resembled that produced by the anti-serum. It is probable that the reaction to injection number two was a reflection of the action of a weak, heterologous platelet agglutinin. The response to injection number four suggested an anaphylactic reaction with autonomic nervous system hyperactivity and intravascular hemolysis, neither of which had been observed on any of the three previous injections.

Figures 2 and 3 depict the responses of dogs which received an injection of the anti-serum. The effective *in vivo* dose was determined to be about 0.17 ml. per kilogram of dog weight. This experiment was repeated many times and never with a fatality. In most instances, the animals appeared mildly agitated for a few minutes after the injection. Occasionally there was vomiting or defecation, but never was there evidence of intravascular hemolysis. No mucous membrane bleeding was detected despite the persistence of platelet counts at levels below 15,000 per cu. mm. for 36 hours. There was no obvious gastrointestinal bleeding, but the stools were not tested for occult blood. Only rarely was the spontaneous appearance of purpura noted. However, on trau-

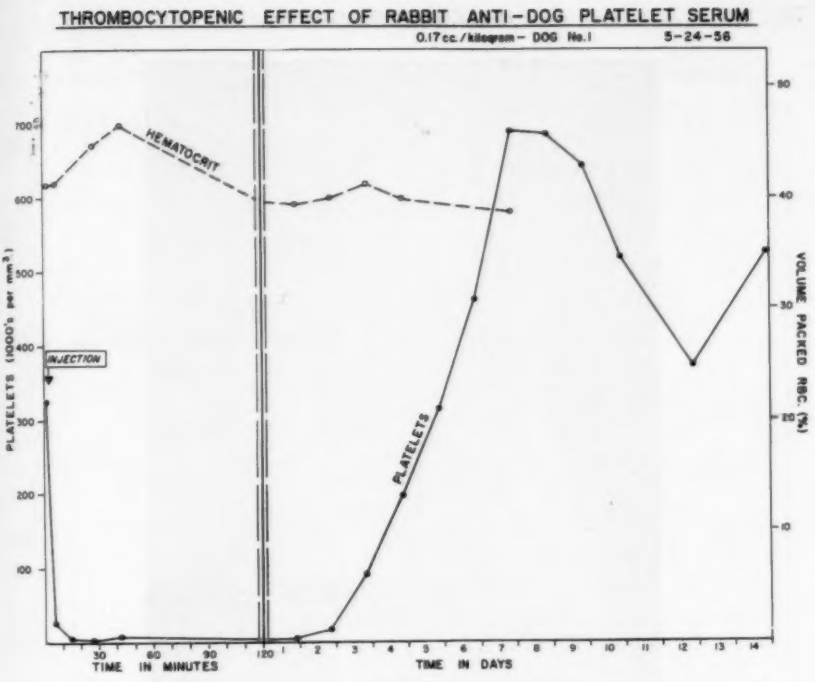
THROMBOCYTOPENIC EFFECT OF RABBIT ANTI-DOG PLATELET SERUM
0.17 cc./kilogram – DOG No. 1
5-24-56
HEMATOCRIT
INJECTION
PLATELETS
PLATELETS (1000's per mm³)
VOLUME PACKED RBC. (%)
TIME IN MINUTES
TIME IN DAYS

FIG. 2

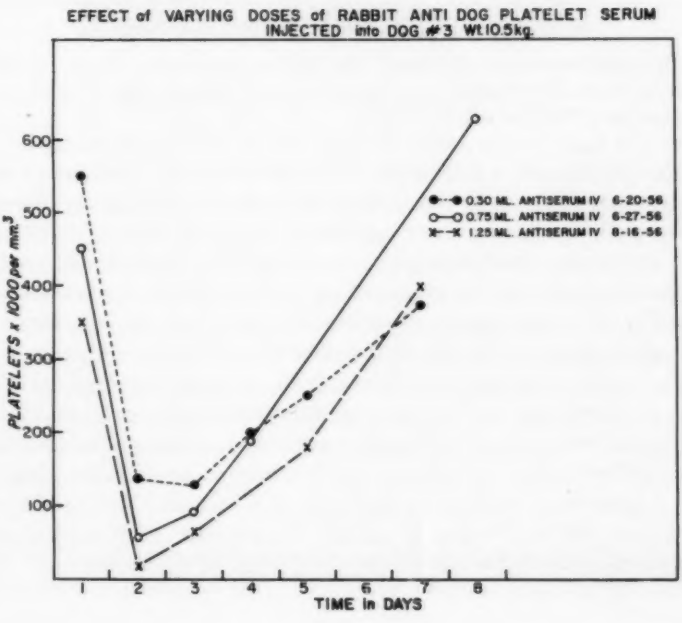
EFFECT of VARYING DOSES of RABBIT ANTI DOG PLATELET SERUM
INJECTED into DOG #3 Wt.10.5kg.
0.30 ML. ANTISERUM IV 6-20-56
0.75 ML. ANTISERUM IV 6-27-56
1.25 ML. ANTISERUM IV 6-16-56
PLATELETS x 1000 per mm³
TIME in DAYS

FIG. 3

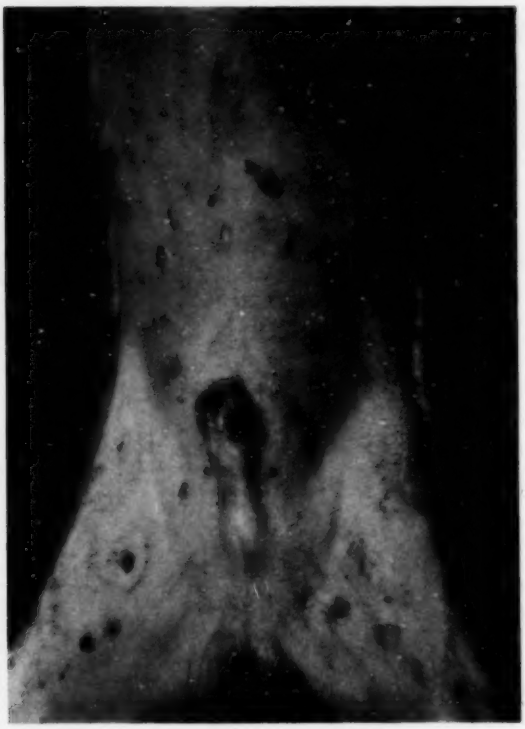

FIG. 4. Appearance of the abdomen of Dog #3 two hours after injection of rabbit anti-dog platelet serum. The platelet count had fallen to <10,000 per cmm. The skin had been traumatized by pinching at sites of purpura.

matizing the tissues of a thrombocytopenic animal, typical purpuric discolorations appeared (Fig. 4). If the animals were anesthetized at the time of the injection of the antiserum there was rarely a noticeable reaction at all. The thrombocytopenic period usually lasted from 36 to 48 hours with a gradual recovery over a 5 to 7 day period. In many instances there was a rebound phenomenon in which the platelet count rose considerably above the normal level, only to return to normal by the 10th or 12th day. In all cases, there was an immediate, transient leucopenia followed by a moderate leucocytosis. Within the first two hours after the injection of the antiserum, there was usually a very slight rise in the hematocrit value. In none of the recipient animals was anemia ever manifested. Injections repeated as often as three times in the same animal produced a profound thrombocytopenia on each occasion. Mongrel dogs which had not been used as a source of platelets for purposes of immunization were as responsive as the donor animals to an injection of the antiserum.

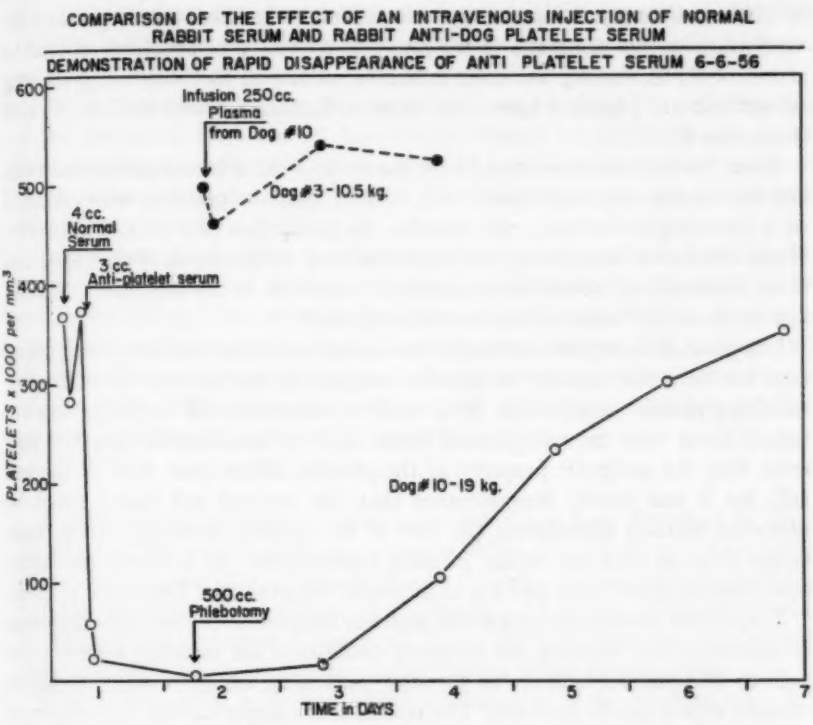

FIG. 5

2. Tests for persistence of the infused antibody

Plasma samples were obtained from the thrombocytopenic dogs at time intervals of 15 minutes, 16 hours, 22 hours, and 48 hours after the injection of the antiserum. These plasma samples, in volumes of 250 ml. failed to produce a demonstrable decrease in the platelet count when transfused into a second, normal dog (Fig. 5). However, this same recipient dog was shown later to be responsive to very small amounts of rabbit anti-dog platelet serum when a direct intravenous injection of undiluted antiserum was made.

The persistence of the antibody after the injection of the antiserum was tested for by *in vitro* agglutination techniques. Serum samples obtained from the thrombocytopenic dogs at intervals as soon as 15 minutes after the injection of the antiserum did not have the capacity to agglutinate *in vitro* test platelets from a normal dog.

DISCUSSION

The production of an anti-platelet serum by immunization with heterologous platelets was accomplished as early as 1906 by LeSourd and Pagniez (5), and

in 1907 by Cole (6). Subsequently, this technique has been used by many investigators as one approach to the study of a variety of problems related to platelets (7, 8). Among the most extensive studies of methods for producing an antibody to platelets have been those of Ledingham and Bedson (9) and Tocantins (10, 11).

From the information derived from the work of other investigators and from the information reported herein, it is evident that the platelet, when utilized as a heterologous antigen, can provoke the production of a potent antibody. From a technical standpoint, the dog serves as a ready source of platelets, and is an ideal animal for *in vivo* experiments inasmuch as the thrombocytopenia can be so readily induced and is so well tolerated.

One great difficulty encountered in the technique for immunization employed here has been the inability to separate completely the red cells from the immunizing platelet suspensions. As a result, a potent red cell antibody was obtained along with the anti-platelet factor. It is of considerable interest, however, that the antigenic property of the platelet differs from that of the red cell, for it was clearly demonstrated that the anti-red cell factor could be absorbed without diminishing the titer of the platelet antibody. If the antiserum is to be used for *in vivo* infusion experiments, the anti-red cell factor must first be absorbed to avoid a catastrophic intravascular hemolytic episode.

Despite the obvious trauma to the platelets incurred in the multiple processes of separation and washing, the antigenic qualities of the platelets were not impaired. The platelet behaves as do most particulate antigens when placed in contact with a specific antibody. The results of the agglutination tests observed in these experiments indicate that the agglutination of platelets can be utilized as an immunologic end point. When the platelets were suspended in native plasma and allowed to react with the antiserum, agglutination was rapid and complete, with few unagglutinated platelets. The reaction was so impressive that it could in no way be termed a questionable or equivocal result. Comparison with the control platelet suspensions left no doubt as to a striking difference. In contrast to the special precautions, complicated techniques, long periods of incubation, and equivocal results that have characterized the attempts of investigators to demonstrate platelet agglutinins in the plasma of patients with thrombocytopenia, the agglutination in these experiments was straightforward, in no way fastidious, and was precise, and reproducible. Any attempt to demonstrate platelet antibodies which produce less than the rapid and unequivocal agglutination described should be viewed with skepticism, when one remembers the capriciousness of platelets and their inherent tendency to clump.

The specificity of the absorbed antiserum and its reproducible effect when injected into the dog make this technique for inducing thrombocytopenia a valuable laboratory procedure. If the appropriate dose is employed, a profound thrombocytopenia is readily induced. No fatalities were encountered in

the course of these experiments. On most occasions, the dogs manifested little reaction to the injection. There was no purpura except at sites of injury, and no mucous membrane bleeding. None of the animals became anemic.

The response to the antiserum was entirely reproducible. After the injection of the antiserum there was an immediate profound fall in the platelet count. The thrombocytopenia persisted for 36 to 48 hours and the platelet count returned to normal on the 5th or 6th day. Blood elements other than the platelets were little affected.

In the amounts injected in these experiments, the antibody very quickly disappeared. Attempts to demonstrate its persistence by plasma transfer and agglutination tests revealed that the antibody could not be detected in the plasma of the recipient animal 15 minutes after the injection. An important aspect of this observation is that these thrombocytopenic animals can be used for the study of the transfusion and survival of platelets or platelet products without interference by persisting antibody.

Summary

A method for producing a platelet antiserum has been described in which rabbits were immunized with dog platelets. When this rabbit anti-dog platelet serum was injected into a dog, a profound thrombocytopenia was induced but without the appearance of purpura or bleeding. There was little alteration in the other formed elements of the blood. It was demonstrated that the antibody disappeared rapidly from the circulating plasma of the recipient dog.

Observations were made concerning the behavior of the platelet when it is used as a particulate antigen to detect the presence of antibody. It was noted that the canine platelets were rapidly and unequivocably agglutinated by the antiserum.

The inadvertent inclusion of canine red cells in the platelet suspensions used for the immunization provoked the production of a rabbit anti-dog red cell factor as well as an anti-platelet factor. The necessity for absorbing this anti-red cell factor prior to infusion studies with the anti-serum was emphasized. It was found that the anti-red cell factor could be absorbed without diminishing the titer of the antiplatelet factor.

Some of the probable non-immunologic causes of platelet agglutination were listed and it was pointed out that platelet agglutination, per se, does not always represent a reaction between antigen and antibody.

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SOME UNUSUAL CASES OF CEREBRAL TUBERCULOSIS

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Despite a constant and gratifying drop in the incidence of clinical tuberculosis, it still resembles the underground guerrilla, always ready to exploit the unwary. The tubercle bacillus has learned to live in a type of symbiosis with "civilized" man, but it stands ready to overwhelm its host after decades of apparent inactivity, and can make its initial attack at any age.

In this country, cerebral tuberculomas are becoming a rarity. Most of the cases encountered are in patients with a known active tuberculous infection or amongst occupants of sanatoria. A rapid, fulminating tuberculous meningitis, while not rare in children, is extremely unusual in adults, even in the presence of a miliary spread. The rapidity with which the infection can produce death is illustrated by the following case history.

Case 1. F. B. A 65-year old man was admitted to the hospital on August 4, 1953 with an illness of one week's duration. He had never had another illness of any consequence throughout his entire life. A few days before admission to the hospital, he developed chills and fever, with some mild discomfort in the epigastrium, followed by nausea and vomiting. He was seen by a local physician who felt that he had an upper respiratory infection and treated him with aureomycin. Vomiting and fever persisted, however, and the patient developed increasing weakness and fatigue. On the morning of admission, the patient collapsed on his way to the bathroom. Following admission to the hospital, he complained of dull bifrontal and bitemporal headaches, which he had not mentioned earlier.

On examination, his temperature was 102, pulse 88, blood pressure 160/100. The patient was lethargic and looked very ill. The skin was dry and he was somewhat dehydrated. The general physical examination showed only some dullness in the left lower chest with a few crackling rales. X-ray of the chest was taken and was thought to show beginning atelectasis of the left lower lobe. A small, densely calcified lesion in the right middle lobe with some calcification at the adjacent right hilum was noted and was thought to be an old, healed tuberculous primary lesion. Despite the use of antibiotics, the patient continued to have fever, became more and more drowsy, and lapsed into pronounced mental confusion. Approximately twelve hours after he was first seen, he developed rigidity of the neck. The patient now appeared acutely ill, with respirations becoming labored and the sensorium continuing to become more and more dull. The following day a lumbar puncture was done. The pres-

sure was not noted but the fluid was cloudy. It contained 600 polymorphonuclear cells and 25 lymphocytes per cubic mm.; the protein was 159 mgm. per cent. It was evident that the patient had an infection involving the subarachnoid system. It certainly did not appear to be the usual bacterial meningitis and the possibility of an acute brain abscess with meningeal reaction was entertained. When seen by the neurosurgical consultant approximately 24 hours after admission, it was noted the patient was having Cheyne-Stokes respirations. He could be roused but was very drowsy. The neck was definitely stiff. The head was not tender to percussion. All extremities moved but there was very little spontaneous movement. All reflexes were present but depressed. There were no pathological reflexes. The optic fundi were within normal limits.

Because of the great decrease in the state of consciousness, plus the difficulty in respiration, it was thought the patient probably had increased intracranial pressure of considerable magnitude and it was decided to do bilateral carotid arteriograms. These were done at once but no abnormalities were noted. On the morning after the lumbar puncture, it was reported that the cerebrospinal fluid sugar was 33 mgm. per cent and there was now a definite pedicle in the fluid. A smear of the pedicle showed acid-fast bacilli. A diagnosis of tuberculous meningitis was made and vigorous treatment was undertaken. Despite the liberal use of isoniacid, para-aminosalicylic acid and streptomycin, the patient's course was steadily downhill and he succumbed on August 14, 1953.

At postmortem examination the lungs showed patchy areas of bronchopneumonia. There were several calicified nodes in the right hilum which were thought to be perfectly benign with no evidence of local activity. Except for the head, the remainder of the post mortem examination was not remarkable. On opening the head, there was evidence of cerebral edema. The cortical sulci were shallow and flattened. Over the basilar portions of the brain there was a thin layer of grayish-yellow exudate, particularly about the pons, brain stem and lower portion of the cerebellum. On the inferior surface of the cerebral hemisphere, as well as the inferior portion of the cerebellum, there were small grayish-white nodules about three to four millimeters in diameter which appeared to be small tubercles. When the brain was sectioned, a large tuberculoma was found in the choroid plexus on the right side. The plexus itself was boggy and necrotic. There was local infectious process going on in the surrounding ventricular wall, also tuberculous. A marked ependymitis of the ventricles was evident. All this appeared to be of relatively acute origin, together with a hemorrhage that was noted in the choroid plexus. The meningitis and small tuberculomas noted on the under surface of the brain and over the hemispheres were probably a secondary spread from the older lesion in the choroid plexus.

Comment: The diagnosis was quite unexpected. Tuberculous meningitis of such severity is most unusual in a 65-year old man with no previous history of tuberculosis. The postmortem findings bear out the contention of many pa-

thologists that tuberculous meningitis is the result of a spread from an established focus in the brain or choroid plexus. It was surprising that such a mass could be harbored asymptomatically in the lateral ventricle for a considerable period of time.

The next patient to be discussed had some features of his illness that made the possibility of a tuberculous infection a part of the differential diagnosis. Other considerations seemed to outweigh this and only after considerable time were we able to confirm the original suspicion.

Case 2. E. H. This 47-year old negro was transferred from another hospital because of extreme lethargy. All the history had to be obtained from the family. His present illness apparently dated from December 1954 when he developed a mild infectious process in the chest and complained of pain in the chest and easy fatigue. Up to this time he had been perfectly healthy, with no complaints of any kind. Family history disclosed that one brother had diabetes and another brother had a tuberculous infection but that there had been no contact with this brother for many years. The patient continued to complain of pain in the chest without any clear reason. He had lost about eleven pounds in weight. In March 1955 he had been admitted to another hospital where physical examination showed only some tenderness at the left costochondral junction. It was also felt that the prostate was hard and that he might have a carcinoma. X-rays of the lung taken at this time showed a slight infiltration of both lower portions of the lungs, thought to be pneumoconiosis. A great number of laboratory studies were done without diagnostic benefit. The white count was 6,250 per cubic mm. with 60 per cent polymorphonuclear cells. X-rays of the skull taken at this time were likewise normal. Because of a tender area at the costochondral junction and the slight thickening demonstrated by x-rays, it was considered to be a metastasis and the area was biopsied. At the time of operation, a necrotic area with local purulent osteomyelitis was found. Cultures taken failed to demonstrate any bacteria. Microscopic study of the area showed only inflammatory cells. The patient was sent home, still with the tentative diagnosis of carcinoma of the prostate. He was given stilbestrol without benefit.

He continued to lose weight and was readmitted to the hospital. This time it was found that he had been having great difficulty in swallowing for a few days. He was confused and it was thought that he might be aphasic. On examination, he was lethargic, pulse was 62, blood pressure 110/80, temperature 100°F. The neck was stiff, although the rigidity was not marked. The remainder of the physical examination still showed only some slight tenderness and swelling over the region of the biopsy on the rib and a firm nodular prostate. There was a beginning hemiparesis on the right with increase in reflexes and a diminution of voluntary movement. Extensive laboratory studies were made without too definitive results. Spinal puncture disclosed a fluid pressure of 200 mm. of

fluid. The fluid contained 27 white cells per cubic mm. The spinal fluid protein was 75 mgm., sugar 56 mgm. per cent, chloride 705 mgm. per cent. A tentative diagnosis of intracranial metastases was made. The patient was referred to this hospital on April 21, 1955.

Upon admission he was extremely lethargic. It was with great difficulty that he could be roused. It was believed here that the prostate was chronically infected and was not the site of a malignancy. Ronchi were heard over both lungs, thought due to an early bronchopneumonia. At the left costochondral margin, at the region of about T-8, there was a healed surgical wound with a little swelling beneath it. The patient now had a rather pronounced right hemiparesis and we wondered whether he was not aphasic as well. Upon examination, the optic fundi proved to be normal.

On questioning the family more thoroughly we got an additional history of an automobile accident in which the patient was involved a few weeks before. X-rays of the skull were repeated and it was thought the patient might have a fracture of the right temporal bone. Since the possibility of a subdural hematoma was likely, it was decided to do diagnostic trephines. These were carried out on the day after admission. There was no evidence for either increased intracranial pressure or bleeding. A ventriculogram was done and the configuration of the ventricular system was within normal limits. Analysis of the ventricular fluid showed 11 mgm. per cent protein, 13 lymphocytes, 91 mgm. per cent sugar, 89 milliequivalents of chlorides. Subsequent bacterial cultures failed to grow any organisms. The patient remained very lethargic and was treated symptomatically. We suspected that he might have tuberculous meningitis but at this point could not verify it. On May 5th a lumbar puncture was done. This time the spinal fluid proteins were 138 mgm. per cent, chlorides 72 milliequivalents, 8 lymphocytes per cubic mm., but the sugar was only 13 mgm. per cent. Cultures were again taken which were sterile but we felt so strongly that the patient had tuberculous meningitis, that isoniacid, streptomycin and para-aminosalicylic acid were started immediately. On the following day after lumbar puncture, a very definite pedicle was seen on the fluid although we could stain no bacteria from the material. Later, dihydrostreptomycin was also given. None of these drugs had any appreciable effect on the illness. The patient developed repeated clonic seizures indicative of midbrain pathology and he had evidences of decerebrate rigidity. Death occurred on May 9, 1955.

Postmortem examination revealed a chronic infection in the left 8th rib at the costochondral junction. Smears of this material showed tuberculous bacilli. There were dense fibrous adhesions obliterating the right pleura due to an old pleuritic infection which had healed. There was evidence of recent bronchopneumonia but no gross evidence of pulmonary tuberculosis. There was a tuberculous abscess in the prostate gland with no evidence of malignancy. On opening the head, numerous grayish-white nodules were found scattered

over the left parietal and occipital lobes. At the base of the brain, along the basilar cisterns and interpeduncular cistern, there was a grayish-white soft exudate characteristic of a chronic basilar meningitis of the type usually seen in tuberculosis. Microscopic sections showed a diffuse miliary tuberculosis scattered throughout both lung fields. The miliary tubercles were generally too small to be seen grossly, which accounted for the "normal" x-ray. Sections of the material of the infected rib showed a picture consistent with tuberculosis. Tubercles were found in the liver and in the prostate. Miliary spread was also found in the spleen and in both kidneys. When the brain was sectioned, the basilar meningitis was quite evident. There was a large, soft area in the insula which was an early tuberculoma. Another tuberculoma was noted in the left basal ganglion. Several small tubercles from 1 to 2 mm. in diameter were found spread throughout the white and gray matter in the midbrain, encroaching on the aqueduct of Sylvius and in the folia of the cerebellum. It appeared that the large tuberculomas in the insula and basal ganglia were older than the small ones that were scattered throughout the brain and meninges. None showed any calcification, however, and it was thought that all the tuberculomas were relatively recent. Two months after death, cultures of the spinal fluid taken in May 1955 were found to be positive for acid-fast bacilli (Figs. 1 and 2).

Comment: While tuberculosis was suspected early in this case, the predominance of cerebral symptoms masked the findings which might have led to a more prompt diagnosis. The head injury reported by the family turned out to be of no importance. The "negative" chest x-ray was likewise misleading. The biopsy, which failed to disclose a metastatic neoplasm, suggested the possibility of a chronic infectious process, finally leading to the correct diagnosis of tuberculosis.

The next case is that of an aged gentleman in whom the onset of a tuberculous central system infection was so insidious that it was months before it was even suspected.

Case 3. R. F. A detailed history concerning the onset of this 70-year old patient's illness was never obtained because the patient himself was too lethargic to give one and his septuagenarian wife was incapable of doing so. We were able to glean from various sources that the patient had been an active worker until July 1956, when he retired. He had been in excellent health for decades. On Christmas day 1956, he suddenly became confused and somewhat lethargic. There followed a steady course of deterioration. He took to his bed with the only complaint of "tiredness". His physician diagnosed one or more "small cerebrovascular accidents". Apparently there were fluctuations in his illness, some days the patient being cheerful, alert and spry, then unaccountably lapsing back into a confused and lethargic state.

He gradually became more and more unresponsive. When first seen by me

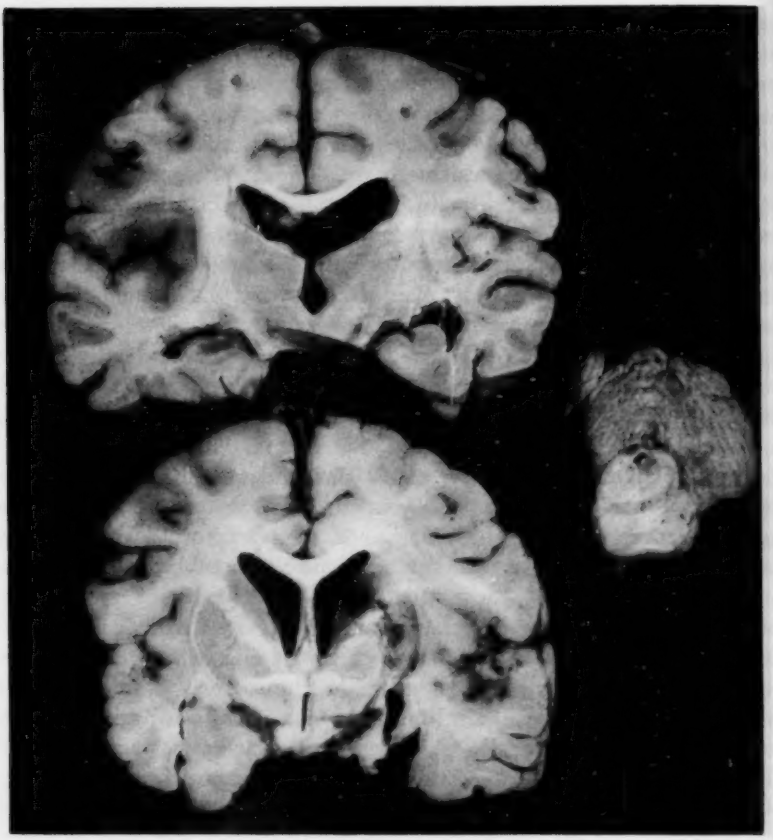

FIG. 1. CASE 2. Coronal sections of the brain. Upper section: Small tuberculomas in the white matter. Lower section: Large tuberculomas in basal ganglia and about insula. Pontine section: Small tuberculoma about aqueduct.

in May 1957, in consultation at another hospital, he appeared to be in a deep sleep from which he could be aroused only by painful stimuli. Confusion and disorientation were evident, but of complaints there were none. Obvious arteriosclerosis and a very stiff neck were the only physical findings. The impaired state of consciousness was the only neurologic finding. The eye grounds showed no evidence of papilledema. X-rays of the chest and skull failed to show any abnormalities. A lumbar puncture done a few days before disclosed a grossly "normal" pressure but 215 mgm. per cent of protein. A perusal of the hospital chart revealed a low grade fever of 100 to 101 degrees, since his admission some days before. Blood studies showed 13 grams of hemoglobin, 14,000

FIG. 2. CASE 2. Microscopic section of tuberculoma found in basal ganglia. Histologic picture is characteristic of a tubercular infection.

white blood cells per cubic mm., with a differential count of 61 per cent polymorphonuclear cells.

The possibility of an infiltrating glioma, a subdural hematoma or a widespread carcinomatosis was considered at that time. Shortly after transfer to this institution, a ventriculogram was done. The brain fell away from the skull. The ventricular fluid was crystal clear and it contained 26 mgm. per cent of protein. Study of the x-ray films was not absolutely conclusive. There was a small defect near the under surface of the trigone of the left lateral ventricle. It was postulated that this might be a small metastasis, an infiltrating glioma, or perhaps just an anomaly.

Because I felt that the small defect hardly accounted for all the findings, very detailed x-ray and laboratory studies were undertaken, without significant results. A repeat examination of the cerebrospinal fluid obtained at lumbar puncture a few days later showed 156 mgm. per cent of protein and 17 lymphocytes per cubic mm. Cultures remained sterile for weeks but on standing for 24 hours, a definite pedicle formed. Since a cerebral tuberculoma with miliary spread seemed quite capable of accounting for the clinical picture, intensive therapy was started, using streptomycin, isoniacid and para-aminosalicylic acid. Despite this formidable array of drugs, plus a high caloric diet administered via nasal tube, the patient failed to show any improvement. He became unrousable, the stiff neck and low grade fever persisted. On the day of death, after six weeks of treatment, he showed an acute change of symptomatology. He suddenly vomited, developed labored respirations and within an hour the right pupil dilated and became fixed to light. Shortly thereafter, the patient died. The final episode certainly appeared to be due to a pontine hemorrhage.

Postmortem examination confirmed the suspicion of a cerebral tuberculoma. A huge tuberculoma was found deep in the left cerebral hemisphere. It was responsible for the pontine hemorrhage, having markedly disturbed the brain and undoubtedly produced severe intracranial pressure changes during the last weeks of life. Small miliary tuberculomas were found in the liver and spleen. An old healed, primary tuberculoma was located in the lungs. Acid-fast bacilli were easily demonstrated in the cerebral tumor (Fig. 3).

Comment: The patient had the more usual course for a tuberculous meningitis, which is slow and insidious. His age and previous good health were factors that lead his physicians to fail to consider this possibility earlier. In retrospect, the clinical course was not unusual, the only unusual feature being the patient's age.

The final case to be discussed is that of an employee of this hospital who had served as a cook for a period of over forty years. He was personally known to most of the staff for years and probably because of his intimate association with medicine, his own problems were not as thoroughly investigated as may have been desired.

Case 4. H.W. A white male, age 65 years, entered the hospital for the sixth time with the complaint of "flu". His first hospital admission was in May 1925 when he entered with the complaints of nausea, vomiting, vertigo, faintness and substernal pain. The typical findings of paroxysmal tachycardia were present. The cardiac rhythm reverted to normal the next day and the patient was discharged. At that time, he gave an account of a similar episode six years before. X-rays of the chest were not obtained at that time.

The second hospital admission was in October 1940, when he was admitted because of a "sore arch" of the left foot of six months duration. A member of the orthopedic department had seen him shortly before and described a

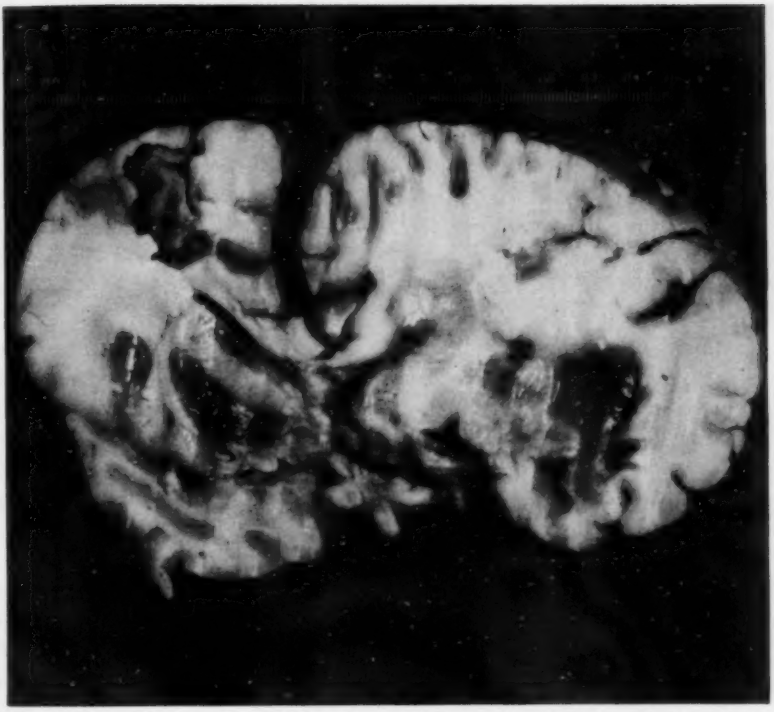

FIG. 3. CASE 3. Large mass of tuberculous granulation tissue in left cerebral hemisphere. Note edema of remaining hemisphere and distortion of the ventricular system.

diffusely edematous left ankle with palpable tenderness over the subastragalar and astragalar-scaphoid region. While in the hospital, no further abnormalities were described. A diagnosis of "arthritis" was made. The ankle was strapped and the patient was discharged.

The third hospital admission occurred a few days later because the ankle remained sore and tender. More searching examinations failed to disclose new findings but there is no mention of an x-ray of the chest having been obtained. Bed rest was prescribed and later a crutch, to walk without weight bearing. The patient was much improved after two weeks of this regimen, and was discharged to return to work. During this stay, there was no elevation of temperature.

The fourth hospital admission was in February 1949, when he entered the hospital to have some varicose veins of the legs treated surgically. Details of this admission are meager but a reference is made that there had been no further "heart attacks" since 1929.

In June 1956, the patient was admitted for the fifth time, because of urinary

difficulties. A hypertrophied prostate was found. No other abnormal physical findings were listed. An x-ray of the chest taken at this time was reported as negative. A transurethral resection was done and his postoperative course was uncomplicated. Pathologic examination of the material removed showed benign hypertrophy of the prostate, as well as "chronic and recurrent acute prostatitis with abscesses". This material has been reviewed in light of later findings and there is nothing to suggest that the infectious process was tuberculous in nature. Since the patient was a hospital cook, periodic examinations were done and photofluorographs of the chest were done in May 1948, July 1949, September 1950, October 1955 and December 1956. All of these films were read as normal.

The final hospital admission was on October 29, 1957, and the patient died nine days later, on November 7, 1957. He entered during a period when the area was having a so-called "flu" epidemic. His complaints were shortness of breath and headaches which had been present for the preceding three days. There may have been some vomiting. Only scraps of history regarding this illness could be obtained because the patient was confused, negativistic, and simply declined to be communicative.

On examination, orientation was slow and it was obvious that he was confused and forgetful. The blood pressure was 120/90, temperature 100.4F.°. There were no other definite physical findings. The neurologic examination disclosed only bilateral Babinski responses. Additional studies included an electrocardiogram, x-ray of the skull, x-ray of the chest, all of which were reported as normal. The fasting blood sugar was 104 mgm. per cent, BUN 20 mgm. per cent, red blood cells 5,300,000, white blood cells 7,450, hemoglobin 17.95 grams per 100 cc., 74 per cent segmented cells and 15 per cent lymphocytes. The urinalysis disclosed a moderate number of pus cells.

A definite diagnosis was not established but it was considered that he probably had the "flu". His temperature persisted at a slightly elevated level. Two days later he was found unconscious in bed. Movements could be elicited on painful stimuli and the neck was rigid. The previously positive Babinski responses were now reported to be negative. Since meningitis was suspected, a lumbar puncture was done. The cerebrospinal fluid pressure was 430 mm. of water. The fluid appeared slightly xanthochromic. Analysis disclosed 10 lymphocytes per cubic mm., 167 mgm. per cent of protein and a negative smear for bacteria.

A neurosurgical consultation was requested because of the persistence of the unconsciousness. At the time, it was noted that the optic discs were within normal limits, all reflexes were depressed and the only pathologic reflex present was a right Babinski. A small healing scab was present on the head and it was speculated whether this was the result of a fall or a blow to the head.

On November 5th, multiple trephines of the skull were done. The brain was

FIG. 4. CASE 4. Large tuberculoma in right cerebral hemisphere, with extension into the ventricle at the region of the trigone, and into the subarachnoid space about the pineal gland and mid brain.

not under pressure. A ventriculogram was done and a generalized dilatation of the ventricular system was found, suggesting an adhesive arachnoiditis. Tuberculous meningitis was suspected and a culture of the ventricular fluid was taken. Three weeks later, a growth of tubercle bacilli was obtained. Analysis of the ventricular fluid showed 29 lymphocytes per cubic mm., 94 mgm. per cent of protein and a negative Wassermann.

The patient's temperature, which had reached 103F.° on November 3d, gradually fell to normal by November 6th. However, there was no improvement in the clinical course and the patient quietly expired on November 7, 1957.

Unfortunately, postmortem examination was limited to the head. The brain was shrunken but over the base of the brain there was considerable thickening of the meninges with adhesions to the medulla, pons, midbrain and chiasmal region. Small pockets of yellow colored fluid were easily seen. The picture was typical of a tuberculous meningitis.

When the brain was cut, a tuberculoma was found in the right cerebral hemisphere, at the level of the pineal gland. It involved the cortex, between the corpus callosum and the medial side of the hemisphere. The mass was about

FIG. 5. CASE 4. Superior surface of the cerebellum showing the area of most severe meningeal reaction.

1 cm. in diameter. The infection then spread to the cisterna ambiens and over the superior, medial side of the cerebellum. This area contained the heaviest concentration of pus. Photographs of the cut sections of the brain are self-explanatory (Figs. 4 and 5).

Comment: In this case, the diagnosis of tuberculous meningitis was not even thought of until the ventriculogram was done. Only then, when the films suggested a chronic adhesive process, was it considered and active steps taken to confirm the suspicion. Once this was done, the diagnosis was easily established. Unfortunately, the postmortem being limited to the head, the primary source of the infection could not be found.

DISCUSSION

Tuberculous meningitis is still fairly common in this country although its incidence is declining, as are tuberculous infections in general. The younger age groups are generally thought to be more prone to this variety of the disease (11), and we tend to forget that it may occur at any age. Also, in adults, meningitis is so often one of the late developments of pulmonary tuberculosis that the latter condition alerts us to the former as soon as symptoms referable to the central nervous system make their appearance.

As has been pointed out by O'Connor (2) the age group of patients admitted to tuberculosis hospitals has been undergoing a great shift. The majority are now over the age of 40 years and 20 per cent are over 60. This has occurred because the younger age groups are being better screened and isolated. The older age groups are not being checked frequently since they are generally missed by such programs as industrial surveys, school screenings, et cetera. Thus they remain a significant untreated reservoir of the disease.

O'Connor (2) also remarks that many of the infections in the geriatric patients are apparently a recent aquisition and not a relapse of an old process. This may perhaps explain the situation encountered in the patients under discussion. Tuberculomas and meningitis are not at all infrequent in the young with recent onset of the disease. The geriatric patient simulates the child in so many ways that pursuing the same course with a tuberculous infection really should not seem unusual.

A great deal has been written in recent years regarding the surgery of intracranial tuberculomas. Excision, followed by intensive antibiotic therapy, is now recognized as the treatment of choice. While neurosurgeons have had much to say about the cases that have been diagnosed antemortem, they write little about those that have not been so localized. In the United States, the incidence of tuberculomas excised in relation to brain tumors is between 1 and 2 per cent. In Chile (3), an incidence of 15.9 per cent is mentioned, in Panama 8 per cent (4), and in North Africa "very high" (5). All these authors point out that the disorder is more common in the "native" population, which, I take it, means in contradistinction to stock derived from European ancestry. Its relative infrequence in the United States, even when the symptoms of an intracranial mass are present, may fail to alert us to the possibility of a tuberculoma before operation is undertaken.

The patients under discussion were all advanced in the course of their disease by the time treatment was undertaken. All had "negative" chest x-rays which delayed the process of arriving at the proper diagnosis. None were operated upon since their principal lesions were relatively silent, both in producing symptoms of intracranial hypertension and in local neurologic deficits. Perhaps excision of the tuberculous masses which must have been spewing bacilli into the cerebrospinal fluid at a vigorous rate might have given the antimicrobial medication a better chance at arresting the infection. The medical therapy undertaken was certainly conspicuous by its inadequacy, which is at variance with the excellent results obtained in youngsters. However, children rarely have sizable intracranial tuberculomas.

While three of the patients reviewed were well advanced in years, they had been in excellent health at the time of onset of symptoms. Post mortem examination failed to disclose any other disease process of significance that

could account for their demise. One must therefore assume that tuberculosis alone was responsible and that the patient's own resistance to the active spread of the disease was poor.

In summary, one can only remark that the possibility of all forms of tuberculosis must be considered in the geriatric age group, even in the presence of "negative" chest x-rays. Treatment must be early and vigorous, and surgical excision of tuberculomas of significant size must be seriously considered, whenever it is applicable.

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ABSTRACTS OF PAPERS PRESENTED BEFORE THE MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

November 10, 1958

STUDIES ON THE TRANSFORMATION OF HEMOPHILUS INFLUENZAE

ROGER M. HERRIOTT, SOL H. GOODGAL AND CLAUD S. RUPERT

STOICHIOMETRY

In order to interpret the stoichiometry of bacterial transformation, it is necessary to know the molecular size of the transforming factor. This was obtained for four different antibiotic factors of *Hemophilus influenzae* labelled with radioactive Phosphorus 32. Sedimentation and diffusion studies at low (0.1–10 μg./ml.) concentrations of transforming DNA led to a molecular weight of 15,000,000 for all components. Since a gram molecular weight has 6×10^{23} molecules, then 1 μg. of DNA, the quantity in 1×10^9 cells, equals 4×10^{10} molecules or 40 molecules per cell. Several experimental results suggest that each of these 40 molecules carries different genetic markers. First, 40–50 molecules of P^{32}-labelled DNA are taken up irreversibly by a population of cells for every cell that is transformed to a particular marker, and this is true for several markers. Secondly, cells resistant to three antibiotics have transforming factors that are independent of each other since the DNA from such cells transform individual cells to resistance to only one or another of the antibiotics. This suggests that the three markers are on different DNA molecules.

IN VITRO PHOTOREACTIVATION

Work of the past decade has shown that viruses, many bacteria, eggs, and even salamanders and white mice can be revived after receiving a lethal dose of ultraviolet light if they are illuminated with sunlight or strong white light. This is known as photoreactivation. Since the experiments suggest that the ultraviolet damage was inflicted on the DNA, an investigation of the transforming factor was undertaken. Preliminary studies on the photorestoration of viability of U.V.-inactivated *Hemophilus influenzae* failed, as did similar studies on pneumococcus. However, when the activity of isolated transforming DNA was reduced by exposure to U.V., and then mixed with extracts of yeast or *E. coli*, cells known to photoreactivate, and illuminated with strong white light, the transforming activity rose from the residual 1 per cent to 30 per cent of the original activity. No restoration occurs unless all components are together at the moment of illumination. The active agent in the extracts is a nondialyzable, heat-labile catalyst and is presumably an enzyme. Its purification is now in progress.

PHOTOREVERSIBLE U.V. LESIONS ON NON-TRANSFORMING HETEROLOGOUS DNA

Heterologous DNA (from phage, *coli*, or thymus) added to the photoreactivating system has no observable effect unless the DNA had previously received U.V. irradiation. In the latter case, the rate of photorecovery of transforming DNA was reduced to 50 per cent when the heterologous DNA was present in equal quantity and when it had been given the same U.V. dose as the transforming DNA. When the U.V.-treated heterologous DNA was given a photoreactivating treatment prior to its being added to the photoreactivating transforming system, it had no inhibiting action. RNA, Kornberg's adenine-thymine polymer, or DNase digested DNA do not exhibit this property, but boiled, acid-treated, or

sonically disintegrated DNA retain the structure in which the U.V. lesion develops. Taken all together this suggests that a photoreversible lesion is inflicted in many kinds of DNA by U.V. irradiation and that competition for the photoreactivating enzyme is dependent on the presence of this lesion, not on the source of the DNA. The present system provides a quantitative assay for these lesions in heterologous DNAs.

GLUCURONIC ACID CONJUGATION BY PATIENTS WITH FAMILIAL NON-HEMOLYTIC JAUNDICE AND THEIR RELATIVES

Barton Childs, James B. Sidbury, Jr. and Claude J. Migeon

This paper in Press.

STUDIES OF GENETICAL LINKAGE IN MAN

J. H. Renwick

The following are some of the uses and possible uses of linkage studies in man.

1. To provide basic cartographic data on the human chromosomes. Gene mapping started for the X chromosome with the color blindness and hemophilia loci (1) and, for the autosomes, with the linkage between the Lutheran blood group locus and the ABH Secretor locus (2).

2. As a tool for detecting genetical heterogeneity in a single clinical entity. For example, it has been proposed (3) that ovalocytosis is due to either of two genes at separate loci, one closely linked to Rhesus (4) and the other not linked to Rhesus.

3. For suggesting that mimic genes are unlikely in a given condition, so that the condition can be used, for instance, in studies of mutation rates per locus. An example is the nail-patella syndrome which shows an apparently consistent linkage with the ABO locus in 14 families (5).

4. For testing for allelism, particularly between two genes so rare that the double heterozygote is not available for study. Linkage of the two relevant loci to a third locus would favour allelism (or close linkage). This could profitably be tried with hemophilia A and B using color blindness as the third locus.

5. For demonstrating non-allelism as in the work of Lees *et al.* (6). They showed that the ectronychia and nail-patella syndromes, though both causing nail and bone anomalies, were not controlled from the same locus, since only for the nail-patella syndrome was the locus closely linked to the ABO locus.

6. To trace an abnormal gene in a family by following a gene known to be closely linked to it. Such a technique is not of general usefulness but has been employed to identify female hemophilia carriers by studying the inheritance of color blindness in the family.

7. Other uses. There will probably be many more demands for linkage information. For instance if a haploid characteristic, say an antigen, could be identified in sperm thus opening up the way to selective destruction or separation, knowledge of the linkage relationships of genes might prove invaluable. However, so far only the four linkages already referred to are really fully established and there are many thousands of genes to plot.

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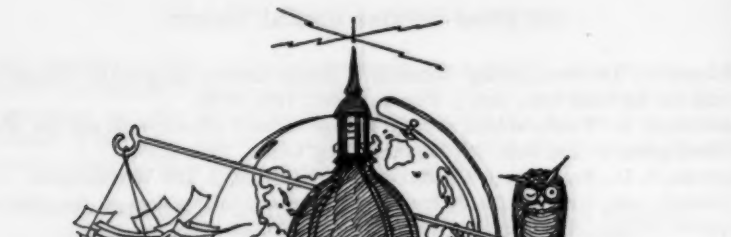

BOOK REVIEWS

La Malattia Microdrepanocitica. By E. Silvestroni and I. Bianco. With a preface by Professor G. Di Guglielmo, cloth, 138 pp., with 36 illustrations and 3 tables. Rome: Il Pensiero Scientifico Editore, 1955. Lire 1800.

This excellent short monograph (in Italian) on sickle cell-thalassemia disease, or microdrepanocytosis, summarises the authors' experience, and reviews the literature up to 1955. It includes material that has previously been published by the authors in English (Blood, 1952, **7**, 429, and 1955, **10**, 623). As Professor Di Guglielmo points out in the preface, Silvestroni and Bianco have a unique experience of the disease, having first characterised it in 1945, and by 1955 having studied some 64 cases of the more than 90 then recorded. Most of their patients were found in Sicily and southern Italy, but others have since been reported in the United States and elsewhere in individuals of Mediterranean ancestry and in negroes (see, for example, Conley, C. L. and Smith, E. W., Tr. A. Am. Physicians, 1954, **67**, 261).

Although an uncommon disease, sickle cell-thalassemia holds great fascination for the general physician, the hematologist, and the geneticist. It results from the coexistence in one individual of two non-allelomorphic genes, that for thalassemia (microcythemia), and that for the sickle cell trait (drepanocytosis). In this doubly heterozygous condition, the genes appear to interact to produce a severe anemia, which neither could produce, acting alone. The clinical manifestations described combine many of the features of sickle cell anemia and thalassemia major, and although quite variable in severity, the disease is often at least as grave as either of these two homozygous states, many affected individuals dying in infancy and childhood, in the authors' series. In the negro, in contrast, the disease is usually considerably less severe, and this difference requires explanation, perhaps in terms of heterogeneity of the thalassemia gene.

The symptoms are perhaps more reminiscent of sickle cell anemia, including episodes of acute pain in the trunk and limbs, fever, and fluctuating jaundice, on a background of chronic hemolytic anemia. In addition to pallor and variable jaundice, hepatosplenomegaly is very marked, and in contrast to sickle cell anemia, splenomegaly frequency persists into adolescence and adult life. Examination of the blood reveals moderate anemia, hypochromia, and wide variation in erythrocyte morphology, with sickle cells, target cells and marked anisocytosis and poikilocytosis. The appearance of the blood smears, and the gross sickling demonstrable, are nicely illustrated. The authors describe this clinical picture vividly and in detail, and there are photographs of many of their patients. They might, however, have

added to the clinical interest if they had been able to include a few detailed case histories with hematological data on individual patients.

Hemoglobin analysis is mentioned very briefly; exact diagnosis in their studies depending upon the methods of classical hematology, and on careful family investigation. Other authors have pointed out that hemoglobin analysis may be most valuable in making this diagnosis, since there is a distinctive hemoglobin pattern, the components being S + A + F, (in quantitative order, as determined by paper electrophoresis and alkali denaturation). Although in sickle cell trait alone the proportion of S hemoglobin does not exceed 50 per cent, in sickle cell-thalassemia disease, as in other sickle cell trait interactions, there is usually considerably more than 50 per cent of S hemoglobin. This apparent intensification of the sickle trait may partly explain the much greater tendency to sickling, and hence the occurrence of severe anemia and acute episodes of infarction, etc.

The chapters on genetics and geographical distribution are of particular interest. In pedigrees in which one parent carries the thalassemia trait and the other the sickle cell trait, according to Mendel's law, the offspring should comprise ¼ sickle cell-thalassemia disease, ¼ thalassemia trait; ¼ sickle cell trait; ¼ normal. This theoretical expectation (using the Weinberg formula correction for small families identified through an affected propositus) is very close to that observed in 26 such pedigrees illustrated. Another striking pedigree (No. 9) of 12 children from a father with sickle cell-thalassemia and a mother with the thalassemia trait, alone serves well to demonstrate the genetic principles.

Since, as the authors show, the disease is not uncommon in the eastern part of Sicily, then the sickle cell trait must also be quite widespread in this region, and indeed Silvestroni has observed sickle cell anemia in Sicily and Calabria. The authors raise the most interesting question of how the sickle trait came to exist in this part of the world (or in any other non-negro population), and offer two alternative explanations. The one which has always been invoked previously is that of repeated sporadic admixture of negro ancestry among the local population in the past. This view has ample geographical and historical justification. Hannibal no doubt brought more than elephants to Sicily! The other explanation offered is the possibility of multiple independent mutations in various non-negro populations. The recent descriptions of foci of sickle trait in many parts of the world, including Sicily, and also Greece, Algeria, and India, may be thought to support this conception. The question should become clearer as the results of current population surveys for sickle trait and other hemoglobinopathies become available.

The authors discuss an important public health consideration, namely that these hereditary blood disorders may be partly preventable by "pre-matrimonial prophylaxis". However desirable this may be, they feel that it is still far from being a practical possibility.

The monograph includes a useful summary in Italian, French and English. There is a list of 87 references, with full titles.

ROBIN M. BANNERMAN

Diagnostic Bacteriology, 5th edition. By ISABELLE G. SCHAUB, M. KATHLEEN FOLEY, ELVYN G. SCOTT AND W. ROBERT BAILEY. 338 pp., $4.75. The C. V. Mosby Co., St. Louis, Mo.

The latest edition of this hardy perennial embodies several new features, the chief of which marks the participation of two additional authors. The latter have assumed responsibility for the new edition and have seen fit to add additional material without introducing any substantial revision of the general organization or format in a book which has previously received wide acceptance. A new section on the identification of fungi associated with systemic infections, and a rewriting of the section on the identification of Gram-negative

rods, represent the more substantial changes in the new text material. In addition, methods have been presented for the identification of the newly recognized strains of *E. coli*, which may be of possible pathogenic significance in infantile diarrhea. The genera *Leptospira* and *Listeria* have also been introduced, as have been the procedures for evaluating sensitivity to bacitracin and optochin, presumptive measures for the identification of Group A beta hemolytic streptococci and pneumococci.

The sections of the book pertaining to bacteriologic procedures reflect the intimate experience of the authors with this aspect of laboratory technology. As such, this volume remains as an authoritative expression which has provided much assistance and comfort to physicians and other workers interested in mastering the techniques used for the isolation and identification of some of the more prevalent pathogenic microorganisms. The need for a volume of this type persists, and perhaps assumes even greater urgency at the present stage in the development of microbiology. The rapid advances in other areas of this discipline have tended to overshadow, if not minimize, the attention paid to the training of medical microbiologists, well versed in the field of "diagnostic bacteriology".

The value of this book might have been enhanced by a more complete and timely selection and critical presentation of the immunologic methods employed as diagnostic aids.

A. G. OSLER

Pathophysiology in Surgery. By JAMES D. HARDY. 704 pp., $19.00. The Williams & Wilkins Co., Baltimore, Md.

Many of the recent advances in the field of surgery and its specialties have been the result of an application of basic physiological principles and their correlation with pathological processes. In this new text one finds an outstanding and selected accumulation of material which clearly demonstrates this relationship in the understanding and management of the surgical patient. It must be admitted that in the wide field of surgery today it is not feasible or even desirable for standard texts to include detailed discussions of physiology. A place is thus created for a reference work designed to fill this need, and it is fortunate that this new volume is now available.

In reviewing the text one is particularly impressed with the fact that a work of this scope has been prepared by a single author. This is most desirable and permits a concise presentation of the material in a unified style and eliminates needless repetition. Each chapter is well written and the bibliography is a carefully selected one. Some of the newer aspects in the field of surgery are also emphasized by the assignment of a full chapter. These include "Radiation and Radioactive Isotopes", "Surgical Enzymology", and "The Physiology of Connective Tissue, Wound Healing, and Homotransplantation of Tissues". Although all parts of the text are of high caliber, probably the most outstanding sections are those devoted to "Body Constituents: Water and Solids", "The Endocrine System", and "Burns". This is logically explained by the author's own interest and recognized investigation in these important aspects of modern surgery.

Without question "Pathophysiology in Surgery" is a new text which can be highly recommended to all students who desire a reliable reference work which correlates basic physiological and pathological principles in the field of surgery. Finally, the volume is also a valuable guide for the teacher of surgery. It is unquestionably the leader in its field.

DAVID C. SABISTON, JR.

Emergency Treatment and Management, 2nd edition. By THOMAS FLINT, JR. 539 pp., $8.00. W. B. Saunders Co., Philadelphia, Pa.

This book is written for the physician who is seeing patients in an emergency department.

It presents its material, both diagnostic and therapeutic, in telegraphic form. Though it concerns itself primarily with common emergencies; i.e., trauma, severe acute illnesses, coma, shock, poisonings, etc., considerable material is included on non-emergency illness such as common cold, chicken-pox, and poison ivy. The medicolegal aspects of handling acute emergencies is discussed at some length.

The author is commended for his selection of conservative, adequate therapeutic measures throughout. Unfortunately, by simply listing them one, two, three . . . no emphasis is given to the mainstays of therapy. The use of morphine, oxygen, and phlebotomy in acute pulmonary edema is excellent, but one may question the equal value of atropine, coramine, and rapid digitalization.

The criticism of this book is not what the author has written but the advisability of writing such a volume. Emergency therapy, regardless of the problem, is based on the dictates of experience and common sense. No book can supply this. This reviewer is still close enough to his accident room experience to realize that very few doctors ever see an emergency patient and then flip through the pages of a book for help in management.

RICHARD REYNOLDS

Infectious Diseases of Children. By SAUL KRUGMAN AND ROBERT WARD. 340 pp., $10.00. The C. V. Mosby Co., St. Louis, Mo.

Not for over a decade has a single volume dealing solely with the infectious diseases been published. Consequently, the main sources of information on this subject have of necessity been either the standard textbooks of pediatrics, which rapidly become outdated in such an active, fast-changing field, or various specialized medical journals, which are not readily accessible to practicing physicians. This book should go a long way to fill the gap, particularly if occasional revisions are contemplated by the authors to keep it up to date. The subject matter covers not only the usual infectious diseases, but there are excellent concise chapters on newly discovered viruses of clinical significance, such as the adenoviruses and the Coxsackie viruses. Each disease, or group of related diseases, is covered in a concise but complete manner with no waste in words or space.

The format is pleasing, the schematic illustrations of various exanthematous diseases are helpful, and there are a number of excellent colored and black-and-white plates. There is also a helpful chapter by Dr. Morris Greenberg of the New York City Department of Health, on the control of communicable diseases.

This is not a book for the research worker or the expert on infectious diseases, but such readers were not in the authors' minds. As stated in the Preface, "It is written primarily for pediatricians, general practitioners, and medical students who deal with children". It fulfills this purpose admirably, and will be a valuable addition to the medical libraries of such members of the profession.

JOHN L. PECK

Clinical Orthopaedics No. 11. Edited by ANTHONY F. DEPALMA. 257 pp., $7.50. J. B Lippincott Co., Philadelphia, Pa.

Volume 11 of *Clinical Orthopaedics* is very similar in style to the previous numbers. However, this issue is disappointing as it was scheduled to contain a treatise on orthopaedic surgery in the senile patient. One could hardly consider the few pages of Section I as even superficially covering this important subject.

Many of the chapters on general orthopaedics in Section II were of limited value. Chapter 22 on "Anatomic Evaluation of Whiplash Injuries" contains, for the most part, speculation concerning the possible causes of pain in this poorly defined condition for which the authors

present no evidence from original work or even cite their references. Rather than decrease the number of patients trying to collect unfair settlements, this sort of writing would appear to help the unscrupulous to develop a case.

One of the most frightening articles is on a "New Method of Pelvic Fixation" which consisted of a large series of pins going into the pelvis which should be capable of producing a large number of operative complications. The few case reports would hardly recommend it.

There are a few worthwhile articles, but there is no uniformity. The quality of printing and binding is excellent.

WAYNE O. SOUTHWICK

Medical Electrical Equipment. Advisory Editor, ROBERT E. MOLLOY. 312 pp., $15.00. Philosophical Library, New York, N. Y.

The preface opens with the sentence: "This book has been prepared in the belief that there is a real and urgent need for authoritative information on the principles, operation, care and routine maintenance of medical electrical equipment, presented in terms that do not presuppose a deep knowledge of electricity".

This general aim has been well carried out by twenty-one contributors discussing subjects from the construction of anti-static floors and room lighting design to circuit details of electroencephalograph amplifiers.

The principal limitations of the book lie in the fact that it deals with specific instruments most of which are of European manufacture and are in limited use in this country.

Useful aspects of the book could be the explanations of the basic principles of operation of various instruments, along with the maintenance instructions and notes on small motors.

GEORGE N. WEBB

Orthopedic Diseases. By ERNEST AEGERTER AND JOHN A. KIRKPATRICK, JR. 602 pp., $12.50. W. B. Saunders Co., Philadelphia, Pa.

I have read this book from cover to cover, and it is very worthwhile. The authors (one a pathologist, the other a radiologist) present the material on an embryological framework which is refreshing; and they add the latest concepts of intercellular components and cellular morphology to advance toward a more truthful explanation of musculoskeletal diseases and disturbances.

Some of their writing is not clear, such as the hereditary, congenital and familial factors, the incomplete treatment of osteoarthritis and the neuropathies, the description of slipped femoral epiphyses and hamartomas. Their attempt to clear the classification of tumors fails, but they are honest and freely indicate there is much to learn.

This book will be useful to all students of the musculoskeletal system, pre and post 'board' orthopaedists, pathologists and radiologists. It is timely with a nice arrangement of chapters and a rich bibliography. I hope the authors will have many editions, each one revised closer to truth and clarity.

W. RICHARD FERGUSON

Cardiovascular Collapse in the Operating Room. By HERBERT E. NATOF, AND MAX S. SADOVE. 197 pp., $6.00. J. B. Lippincott Co., Philadelphia, Pa.

The spectre of cardiac arrest during surgical procedures is continually in the presence of the surgical team during an operative procedure. Cardiovascular collapse has been a subject for many and endless discussions, especially in the past decade. One's own experience points up the fact that more often than not, possible causes leading to cardiac arrest are extremely difficult to document, and autopsy findings do not yield any helpful information. The authors

of this fine monograph have taken all of the instances of cardiovascular collapse which have occurred at the University of Illinois Research and Educational Hospital in the previous three years and have analyzed them in an effort to find the possible causes of cardiac arrest. As a background for their own study, they have made an extensive analysis of operating room morbidity and mortality data from 1920 to 1956. This analysis pointed up the lack of uniformity and accuracy of the data. They stressed the need of the establishment of a uniform methodology for collection, classification, and interpretation of operating room morbidity and mortality data to insure a better understanding of cardiac arrest. The analysis of their own cases and the data made available to them since 1920 indicated strongly that there was nearly always a multiple predisposing and precipitating factor for cardiovascular collapse. Their own cases are very well documented and classified according to causation. There is an excellent chapter on the possible mechanism of cardiovascular collapse, and a good portion of the book is given to the aspects of prevention, diagnosis, and treatment of cardiovascular collapse in the operating room.

This is an unusually fine coverage of a difficult and controversial area of surgery. The book deserves careful and thoughtful study by all those who are part of a surgical team and who desire to continuously diminish the occurrence of cardiovascular catastrophe in surgical procedures.

DONALD W. BENSON

BOOKS RECEIVED FOR REVIEW

Amid Masters of Twentieth Century Medicine. A Panorama of Persons and Pictures. By LEONARD G. ROWNTREE. 684 pp., $11.50. Charles C Thomas, Springfield, Ill.

Anatomy for Surgeons, Vol. 3, The Back and Limbs. By W. HENRY HOLLINSHEAD. 901 pp., $23.50. Paul B. Hoeber, Inc., Medical Book Dept. of Harper & Bros., New York, N. Y.

Applied Medical Library Practice. By THOMAS E. KEYS. 495 pp., $10.75. Charles C Thomas, Springfield, Ill.

Bacterial and Mycotic Infections of Man, 3rd edition. Edited by RENE J. DUBOS. 820 pp., $8.50. J. B. Lippincott Co., Philadelphia, Pa.

The Birth of Normal Babies. By LYON P. STREAN. 194 pp., $3.95. Twayne Publisher, Inc., New York, N. Y.

Breast Cancer. The Second Biennial Louisiana Cancer Conference. Edited by ALBERT SEGALOFF. 257 pp., $5.00. The C. V. Mosby Co., St. Louis, Mo.

Cardiovascular Sound in Health and Disease. By VICTOR MCKUSICK. 570 pp., $15.00. The Williams & Wilkins Co., Baltimore, Md.

The Care of the Geriatric Patient. Edited by E. V. COWDRY. 438 pp., $8.00. The C. V. Mosby Co., St. Louis, Mo.

The Chemistry of Blood Coagulation. By PAUL MORAWITZ. Translated by Robert C. Hartmann and Paul F. Guenther. 194 pp., $4.50. Charles C Thomas, Springfield, Ill.

Difficult Diagnosis. A Guide to the Interpretation of Obscure Illness. By H. J. ROBERTS. 913 pp., $19.00. W. B. Saunders Co., Philadelphia, Pa.

Emotional Problems of Childhood. Edited by SAMUEL LIEBMAN. 176 pp., $5.00. J. B. Lippincott Co., Philadelphia, Pa.

Epilepsy. By MANFRED SAKEL. With a Preface by Otto Poetzl. 204 pp., $5.00. Philosophical Library, New York, N. Y.

Handbook of Cardiology for Nurses, 3rd edition. By WALTER MODELL AND DORIS R. SCHWARTZ. 328 pp., $4.50. Springer Publishing Co., New York, N. Y.

The Integration of Behavior. Vol. III, The Reintegrative Process in a Psychoanalytic Treatment. By THOMAS M. FRENCH. 484 pp., $10.00. The University of Chicago Press, Chicago, Ill.

Treatment in Internal Medicine. By HAROLD THOMAS HYMAN. 609 pp., $12.50. J. B. Lippincott Co., Philadelphia, Pa.

Tuberculosis. Prevention and Control. By H. W. HETHERINGTON AND FANNIE W. ESHLEMAN. 404 pp., $6.50. G. P. Putnam's Sons, Including the publications of Minton, Balch & Co., New York, N. Y.

THE STUDY OF THE HISTORY OF MEDICINE[1]

OWSEI TEMKIN

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In recent years, much has been written and more has been said about the teaching of medical history. I do not wish to belabor that topic anew—especially since last year the history of medicine was made a required subject at our medical school. Instead I would like to discuss with you the study of medical history. I shall assume that one *wishes* to study the history of medicine—something a teacher cannot take for granted among his pupils. I shall merely raise the question: in what spirit and where should this be done?

Before this question can be approached, we have to clarify whom we mean by "one". There are many potential students and the task is different for each of them. There are, for instance, the novelist, the playwright—and even the producer of historical films, whom I would ardently like to see a student of medical history. Then perhaps, the day would come when the doctor of the 18th century would no longer pull a stethoscope out of his pocket and, having performed this anachronistic feat, proceed to apply the earpiece to the patient's chest. Such studies could be confined to external appearances and need be little concerned with a deeper understanding of medical conditions and science, which can be left to the historian interested in medicine and to the physician interested in history. It is to these two that I would like to devote the rest of our discussion.

Let us take the historian first. By definition a historian is a person who wants to know the past. He will ask what was done in medicine, by whom, for what purpose, and how? He will soon perceive that these questions cannot be answered without a much wider setting. For instance, the healing art in this country, some 200 years ago, was not as sharply divided into the separate domains of physicians and surgeons as it was in Europe. Under the conditions of a thinly settled continent this obviously could not be the case, and John Morgan failed when, in 1765, he wished to introduce the separation.

As this very simple example shows, the historian must know the relationship of medicine to other conditions of the time. This will make him inclined to break up medicine into the areas of different civilizations and periods. For the older civilizations a thorough study presupposes linguistic knowledge, which is a study in itself. Whoever works on Egyptian or Babylonian medicine without knowing hieroglyphic and cuneiform scripts does so at the peril of dilettantism. For many periods, a knowledge of the times is more important than a technical knowledge of medicine. Dr. Edelstein's (1) work on Hippo-

[1] Paper read before the Johns Hopkins Medical History Club, November 24, 1958.

cratic medicine is an excellent example. While most physicians were fascinated by the medical meaning of prognosis, which predominates in the Hippocratic writings, and tried to read a deep scientific significance into it, Dr. Edelstein found a social motivation. The ancient doctor had to create his clientele and to impress it by announcing the past, present, and future conditions of the patient, and he had to exonerate himself in advance in hopeless cases. For more recent times, I might cite Professor Shryock's (2) *Development of Modern Medicine* as an outstanding example of the successful approach by the general historian.

I by no means believe that the historian should concern himself only with the background, social, economic, cultural, and intellectual, of medicine. In our present context I use the word "historian" to denote anybody who wishes to know how things in medicine were. Obviously, that relates to medical and scientific content, no less than to social environment. What Galen knew about anatomy, what discoveries William Beaumont made about gastric function, what the relative incidence of diseases was and how they were treated at the Johns Hopkins Hospital during the first years of its existence—all these questions belong to the realm of the historian. But whereas a study of the background necessitates, above all, a knowledge of existing general conditions, questions like the above depend on familiarity with medicine and its sciences. This has given rise to a serious problem. A historian who makes medicine the object of his study should know general history and he should know medicine; how can these two be combined?

It is interesting to see the difference between the solutions that were offered formerly, and those offered now. Medical history, until thirty years ago, was undisputedly in the hands of medically trained persons. To be sure, there were some philologists and a few historians who occupied themselves with editions of old medical texts and with ancient or medieval medicine. But they were not medical historians like Sudhoff, Diepgen, Sigerist, Neuburger, Garrison, Singer, Castiglioni, all of whom were doctors of medicine. In those days one became a historian of medicine as one becomes, for instance, a pathologist today. A knowledge of Latin and Greek and of some modern European languages was taken for granted. Either in connection with the thesis required in most European countries for the M.D. degree, or in some other way later on, a connection was established with a professor of medical history. Paid positions being extremely few, much had to be done privately. But with or without a job, one tried to learn such historical methods as were needed for one's research. A humanistic education, a course during student days, supervised work on the thesis, and much private study constituted the main elements in the training of so-called historians of medicine. The result was not altogether satisfactory in every respect; many mistakes were made which a

regular student of history is taught to avoid in his first year. But a tradition was established and maintained: A medical historian was able to understand and, if necessary, teach the history of medicine as a whole, i.e. of all times and in all aspects. He was supposed to be able to contribute to the understanding of the development that extends from Hippocrates to our own days, even if he considered some particular period the province of his more sustained research.

This relatively simple scheme has been upset by several factors: In this country the knowledge of foreign languages in general, and of the classical languages in particular, has declined to a degree that for the average M.D. all but excludes a serious occupation with medicine outside of the Anglo-Saxon civilization. Together with this loss has gone an increasing inability to enter into the spirit of a civilization fundamentally different from ours. The boy who labored over Homer and Cicero for years knew at least one thing: it had all been so very different! Our loss of feeling for such differences has as a result that the philologist and general historian are now taking over what was once done by physicians. Moreover, historical studies themselves have advanced far beyond their erstwhile simplicity. Not only have they acquired a strong sociological element, but they have also extended outside their traditional fields. The social historian encounters medicine as a very important phenomenon: sickness, mortality, and the institutions for their prevention and cure are social factors, to be studied and understood. The medical student obviously cannot cope with the trained young historian—even if he wished to do so. Gradually, the serious study of medical history is being transferred to the general historian. But the general historian is "general" for his particular period only; he is not prepared to deal with medicine of all times. Moreover, he encounters a serious limitation in the subject matter. Thus a division of labor has been suggested: Let the historian look after the social and cultural development of medicine, and let the medical man study the technical developments. I confess that this solution reminds me of Solomon's judgment: it will lead to the death of the child.

So far we have considered the historian who wants to know how things once were in medicine. We have used the term "historian" merely from the point of view of interest—not of schooling. Karl Sudhoff, for instance, was a trained medical man who for many years had engaged in the practice of medicine. But he investigated medicine of the past, particularly of the Middle Ages, as a medievalist would. He tried to find new texts, he deciphered, explained, and published them. For the rest, he was little concerned with their bearing on present or future medicine. In short, he was not a physician who turned to history because he expected from it elucidation of his practical and scientific work.

Lest we overlook the obvious, we must remember that at all times there has been the sheer enjoyment of good stories and anecdotes. This element must be rather strong, judging from the use made of it by advertising. Pamphlets, and even journals, brought out by drug houses are replete with articles and illustrations relating to medical history. This would not be so if history did not hold an attraction for laymen and physicians alike. Such articles vary from excellent historical accounts written by good historians to vulgarizations abounding in mistakes. To be sure, all history has a romantic aspect which may be the element that makes it humanly attractive. But the publications I am now speaking of are not always history. Sometimes they respond to the legitimate desire for entertainment, or to the not so legitimate desire for introduction to the magic of miracle drugs and the working of the modern shaman called "the man in white".

No serious study is involved on the part of the person who wishes to be entertained. But how about the scientists and physicians who wish to consult the past for their immediate concerns? It can be said that the number of scientists who expect from history something other than mere knowledge of the past is growing. There exists, today, an increasing need for orientation in the meaning of fundamental concepts. Unless I am mistaken, physicists have taken the lead, but biologists and physicians are following. Before I came to this country, in 1932, I had done some work in the history of the concept of disease. But in 1932 I did not feel our climate here propitious for that kind of research. By now, the situation has changed. Concept of disease is one example among many where an elucidation of present thinking is expected from the tracing of the historical development. But—and here lies the difference from history as such—the past is studied only in as far as it promises to be helpful. The scientist does not want history; he wants something of history. As an example I may quote J. R. Baker (3), who accompanied an admirably painstaking reexamination of the history of the cell theory by the following remark:

> Though I have great respect for the history of science, yet my main purpose in this series of papers has not been to write history, but to use a mainly historical method to establish what I believe to be an important truth about living organisms.

And when we turn to the doctor who asks of history to keep alive what is best in a tradition and to let the great masters of the art speak, we encounter a similar situation. Since I do not practise medicine, leave alone surgery, I must be forgiven if I try to project myself into the mind of a surgeon. But conversations with surgeons and other practitioners have led me to believe that to many of them thoughts, manners, and even mannerisms of the great teachers have a deep meaning. Halsted is not just a name connected with certain operations. It is a way of approaching surgical problems and dealing with them.

Other great surgeons had different ways. In spite of the fact that medicine has become "scientific" and much has become standardized, it involves a good deal of art. Art is learned by apprenticeship from example, and history is to keep alive the great examples.

I express these thoughts with some diffidence, but I have to enter upon them because they concern the study of the history of medicine. To put it bluntly, they lead to the question whether the study of the history of medicine had not better be left where medicine is taught and practised.

This question is not an idle one, and there is good reason to bring it up at our Medical History Club. Until some 25 years ago, this Club met here in the Hospital where it had been founded in 1890 by Osler, Welch, and Kelly. And until about the same time, the *Bulletin of the Johns Hopkins Hospital* used to carry a considerable number of historical articles, particularly those emanating from the papers presented before this Club by students, members of the faculty, or invited guests. Then, the *Bulletin of the History of Medicine* was founded as an independent organ, and for many years our meetings were held at the Institute of the History of Medicine. Thus, a good deal of the study of medical history left the medical departments and the hospital and migrated to the professional medical historians. The opinion has been voiced that a good deal of interest in the study of medical history left the school and the hospital at the same time.

I wish I could simply deny this. But I have to admit that to some extent I agree with this opinion. Whenever I go through the old volumes of the Bulletin of the hospital, I am impressed by the excellence of so many of the historical papers. I must even admit that our students have ceased to produce the kind of historical papers that their elders apparently were able to deliver. And the fact that we are meeting in the Hospital tonight, shows that the officers of this Club, several years ago, recognized the necessity of dividing our meetings between the Hospital and the Institute.

We thus seem to be confronted by a dilemma. On the one side, we have medical history as studied by the historian with its ever increasing demands upon historical knowledge, paralleled by an ever decreasing mastery of historical tools on the part of medical men. On the other, we have the vital interest of the physician and medical scientist who ask of history what the historian is not necessarily willing or able to give: the link between the present and that which did not die with the past. Does this dilemma require a solution? and if so, where is the solution to be found?

I believe that the dilemma needs a solution, and that this solution will not be found in either of the two extremes. If the history of medicine were to become a mere branch of history, it would not cease to be of interest to medicine. Let us not forget that medicine itself is changing. The time is not very

far past when medicine was the exclusive domain of doctors of medicine. But in the broad sense, this is no longer the case. The licensed practitioner of medicine is on the way to becoming a specialist within the wider social structure which we call medicine. By "specialist" I mean the man who is in direct contact with patients, while an army of other specialists, mainly Doctors of Philosophy or of Science, works in laboratories, libraries, offices, and public health agencies. This specialist role is enhanced in this country by the limitation of the number of M.D.'s turned out by our medical schools. Doctors of Medicine are so badly needed in their practical functions that few can be spared for other work, be it in the basic sciences or elsewhere. At any rate, the changing character of medicine will fortify the demand for historical analysis. Of this I feel sure. However, I believe that the historian who is concerned only with a knowledge of the past will not be able to answer all questions put to him. It is hardly his business to evaluate what was done formerly in comparison to what is being done today. Especially when questions of medical science or practice are concerned, the historian tends to be over-awed by the achievements of modern medicine and to use them as an absolute measuring stick, or to be satisfied with bringing out the fundamental differences between then and now. He will not easily be inclined to use history critically, yet constructively, except in the region of social conditions.

On the other hand it appears equally doubtful that we can go back to the time of Osler and keep historical studies a mere adjunct of the physiologist, internist, or surgeon. In making this statement I would like to head off a misunderstanding. I do not wish to see such studies weakened, leave alone taken away, from the departments. On the contrary, I would do everything I could to encourage them and cooperate with them. The active medical scientist and practitioner has a direct, vital approach to his subject and a grasp on the more recent developments in his field which a so-called medical historian will not be able to emulate. It is here in the departments that our students must gain the feeling that the historical approach is the concern of all of us. Here are the grass-roots of the history of medicine as a part of medicine. If these roots were to die, then as I said before, the history of medicine would not disappear; but it would become a subdivision of general history, looking upon medicine as an interesting object, without having a share in it. In saying that we cannot go back to the days of Osler I meant that these studies by the medical man can no longer be carried on in isolation from the general study of history.

I do not know how far ideas like these were in the mind of Dr. Welch when he planned the Institute of the History of Medicine some thirty years ago. But it may not have been chance that led the first head of our pathology department to become the first director of the Institute of the History of Medi-

cine. Within the present context, pathology and medical history seem to have much in common.

Before the days of Rokitansky and Virchow, pathology hardly existed as a specialty.[2] The clinician performed the autopsies on his cases, possibly helped by a prosector. It was, and still is, said that something was lost when the professor of pathology took over, and that it would be a good thing to compel the clinician to go back to the autopsy table. Even if that were true, pathology was bound to become independent. It had to find its connection with general biology as a natural science. This led to Virchow's *Cellular Pathology*, published just a hundred years ago, and to pathology as an experimental science. It did not mean that pathology lost its autonomy. Today, every medical man studies pathology with a specialist. But the medical man also remains somewhat of a pathologist, regardless of where his particular field of interest lies. Indeed, he may know more of the pathology of a particular organ or disease with which he is concerned than does the professional pathologist. It would be just as impossible to take pathology out of the departments, as it would be foolish to drive it back into the departments. Somewhere, pathology must be studied as a science, even though the pathologist may not be able to compete with all specialists in their particular fields.

Replace biology by history, and the parallel with medical history will be clear. I do not wish to force the analogy; it has many weaknesses and was intended only to indicate the place of the study of medical history as a discipline. This study will have to be pursued in close contact with the general historian as well as the medical scientist and practitioner. This means that a department of the history of medicine should have room for those who wish to know how things once were, and for those who are pursuing historically an actual medical problem. The study of the history of medicine, even towards professional status, should not be made dependent on the degree the student holds. The general historian with his Ph.D. degree or candidacy for such a degree, and the Doctor of Medicine, or medical student, each has his advantages and handicaps. A department of the history of medicine should be prepared to help either of the two in overcoming obstacles. As far as the medical man is concerned, this is best done by apprenticeship. Those of our medical students who have serious historical interests should be encouraged to try doing historical research work. Medical graduates may even study towards a Ph.D. degree, though I would recommend such a formal path to professional aspirants only. If a department has medical men working in it, their very presence will facilitate the path for the general historian, or sociologist. In contact with them, he may learn what books alone cannot supply, an impression of the

[2] In a conversation with Professor Shryock I was reminded of the significance of pathology's early struggle for independence.

spirit of medicine. The position of the department within the medical school may then also enable him to establish contact with other medical departments. It goes without saying that his contribution in showing the medical student the methods of historical research can be very great.

All such arrangements are viewed from one central point: the pursuit of medical history as a subject combining in itself historical and medical interests.

It seems to me that this combination of interests should characterize the medical historian, in distinction from the pure historian dealing with medicine, and from the active medical scientist using history. That the borderlines on both sides will not be sharply drawn, goes without saying. If I speak of combination of interests, I do not have in mind their mere addition. Elsewhere, I have tried to show how an organic union between historical and scientific research might be achieved (4). Tonight I am dealing with "study", which is more comprehensive than "research" and concerns a wider audience.

To sum up: the history of medicine can be studied as mere history; it can also be approached with a medical and scientific interest. Both approaches have their right to existence, both can lead to valuable results, and both should be cultivated. But beyond either of them there should be an organic synthesis which we may call the history of medicine *per se*. The existence of such a discipline justifies the existence of independent departments of the history of medicine. Here at Johns Hopkins we are privileged in having an Institute with excellent physical facilities, situated in a great medical library; we are linked with a philosophical faculty with a strong humanistic orientation, and we are part of a medical school famous throughout the world. Such a privilege imposes a duty: the duty to see to it that the spirit of the place and the work done in it be equal to the task and worthy of the place and its great tradition.

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A COMPARATIVE STUDY OF THE VERTEBRATE NASAL CHAMBER IN RELATION TO UPPER RESPIRATORY INFECTIONS

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PART I

COMPARATIVE MORPHOLOGY OF THE NASAL CHAMBERS OF THREE COMMONLY USED LABORATORY ANIMALS: CHICKEN, RAT, AND FERRET[1]

"Il est rare qui'il soit advantageux de se refugier dans le monde de la hypothese. Il y a dans les annales de la science plusieurs examples qui montrent comment les plus belles hypotheses ont ete renversees a la suite d'une seule exception qui se base sur un examen anatomique."

Louis JACOBSON, 1811
Ouvrages sur L'organe Vomero-Nasal

When infectious agents are introduced into the nasal chambers of experimental animals, a series of reactions is initiated. For interpretation of these responses to disease, an understanding of the normal structures and their functions is prerequisite. In studying normal prototypes of three laboratory animals commonly used for experimental respiratory infections, we have been handicapped by being unable to fully identify some of the intra- and paranasal organs, so that the possible role of these organs in defense against or susceptibility to disease has not been explicit. It has therefore seemed useful to study the organs which discharge fluids into the upper tracts of these representative types in order to better evaluate the pathology. Using the chicken, rat, and ferret as principal models we have begun a systematic study of vertebrate upper tract structures as they relate to the normal function and defensive resources of the nasal fossa.

There are many excellent accounts of the comparative morphology and evolution of the vertebrate organ of smell (1–10)[2] but studies oriented toward normal and pathological tissue function are either scattered in specialized literature or apparently unavailable.[3] We would like therefore to report (a) a study of the gross and microscopic anatomy of the three familiar animals, and (b) a review of the literature in experimental biology, phylogeny, and physiology as applied to the vertebrate nasal chamber. We have not included at

[1] The comparative morphological study was supported in part by the Lasdon Foundation.

[2] References are listed at the end of Part II.

[3] The valuable studies of Hilding (63), Negus (87), Nemours (86) and others on nasal comparative anatomy and physiology have emphasized comparative principles of clinical disease; our effort is rather to relate the biology of upper tract disease to the adaptive variation of homologous organs.

this time the very pertinent literature on lymphatic drainage (122) or on the electron microscopy of intranasal tissues (123–125).

We want to make it quite clear that we have simply observed the structures that are described. At the same time we have tried to find what is known concerning their function. For purposes of clarity we have included some of this information with the descriptions of our specimens, though most of it is alluded to in fuller context in the review. Also, we have acquired a few wild forms and zoo specimens which are used both in the anatomical study and in the review to illustrate points at hand.

MATERIALS AND METHODS

Specimens of freshly killed young adult New Hampshire Red chickens, Brown Norway rats, common British and common American ferrets were fixed for 48 hours in Witmaack's solution, then for 2–10 days, depending on the animal's size, in Orth's solution, and then decalcified in either 1 or 2 per cent nitric acid solution until the cranial bones were amenable to slicing with a razor blade. They were then neutralized in 5 per cent sodium sulphate solution and rinsed overnight in running tap water. One specimen of each animal was used for gross study and one for microscopic when possible. Gross serial sections were done by hand at about 2 mm. intervals. Vegetable shortening was packed into the nostrils and the specimen was refrigerated until the shortening was firm; the position of the soft tissues was then not distorted by sectioning. The shortening was easily rinsed out with warm water after a section was sliced with a razor blade. Microscopic preparations were dehydrated in graded solutions of degassed distilled water and ethanol and embedded in either celloidin or paraffin blocks and stained usually with hematoxylin and eosin. Copper chrome hematoxylin was used for some of the ferret sections, and iron hematoxylin for the smaller of the coccobacilliform-infected chickens.

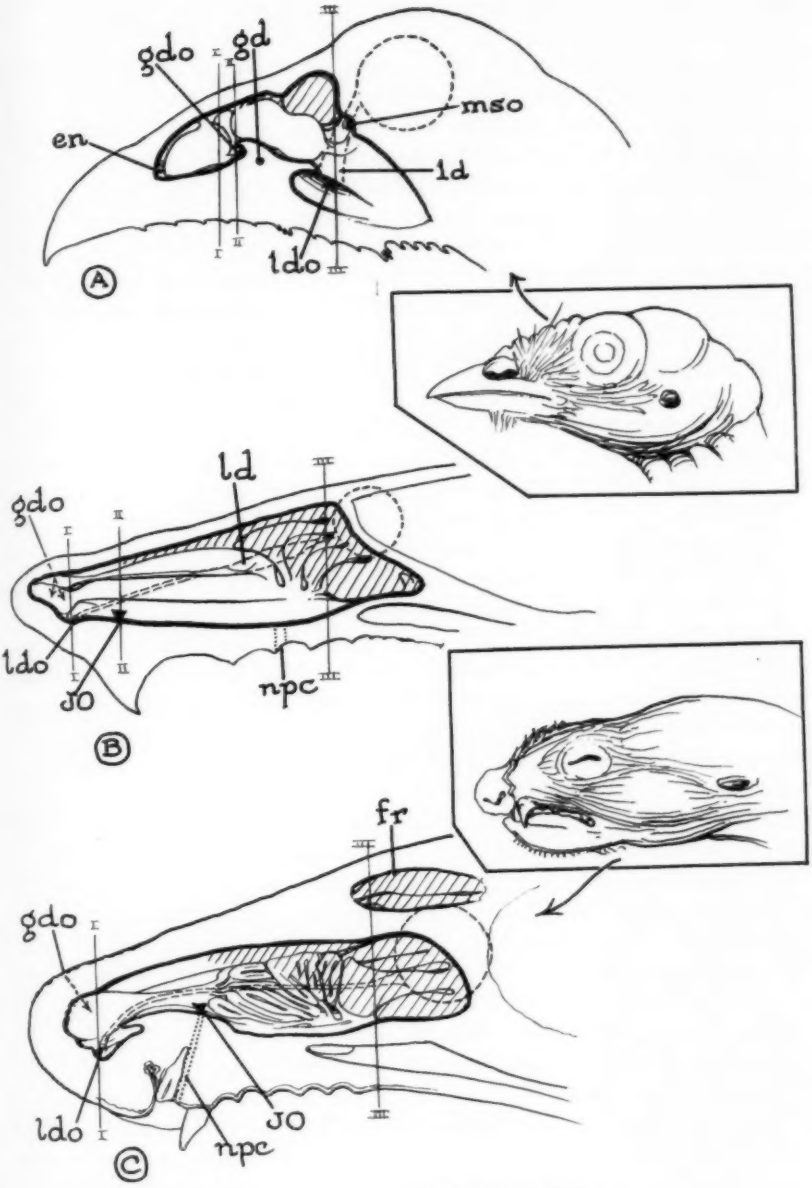
Orthometric drawings were made of each gross section, at magnifications of 10 diameters for the chicks and usually 20 diameters for the chickens and mammals, using a Zeiss binocular dissecting microscope. These drawings, in combination with the microscopic sections, were used as the basis of serial diagrammatic maps prepared with colored pencil crayons in which tissue types could be uniformly symbolized. Some of these have been translated into line drawings in the accompanying figures.

Some of the wild-caught specimens were preserved in 10 per cent formalin or in Ringer's solution in the field; most of these were not found suitable for histological study, but were decalcified and then kept in a preparation of 5 parts of phenol, 30 parts of glycerin, and 5 parts of 10 per cent formalin in 60 parts of water, and sectioned or dissected.

COMPARATIVE ANATOMY OF THE CHICKEN, RAT, AND FERRET NASAL CHAMBER

In so far as it is feasible, we will consider the structural common features of the three types, the adaptations apparent for each type, and the common functional unknowns. These include the maxillary sinus and other incursions

FIG. 1. Diagrammatic Sagittal sections of right medial surface of nasal chambers of chicken (A), rat (B), and ferret (C) with septum removed. Not drawn to scale. Olfactory sensory area indicated by diagonal shading, position of eye and lacrimal duct by broken lines. Inserts of partially dissected heads of chicken and ferret give idea of extent of fossa in relation to external landmarks. Small Roman numerals and fine vertical lines show level at which vertical sections in figs. 2 (I), 3 (II), and 10 (III) were made.



en = external naris
fr = frontal recess
gd = lateral nasal gland duct
gdo = lat. nas. gland duct orifice
jo = Jacobson's Organ, site of opening
ld = lacrimal duct
ldo = lacrimal duct orifice
mso = maxillary sinus ostium
npc = approximate position of naso-palatine canal

into the bony skull; the two chief compound intranasal gland groups (the lateral nasal and the maxillary) and their efferent ducts; the lacrimal duct; the vomeronasal organ; the oronasal connections; and some of the differences in the relationships of these structures. Figure 1 shows some of the comparative features diagramatically in sagittal views of the medial wall of the right nasal chambers of the three species, the septum being removed but the position of the vomeronasal orifice indicated. Insets of partially skinned heads of the chicken and ferret are included to orient the relation of the nasal chamber to the external landmarks. The extent of the olfactory epithelium in this plane is indicated by diagonal lines. The positions and relationships of the olfactory area, the mouth of the vomeronasal (or Jacobson's) organ, the efferent duct systems of the lateral nasal glands and lacrimal fluids, and the connections between the oral and nasal cavities by way of the nasopalatine and choanal passages are also shown. Structures which lie between the mucosae and the bony nasal capsule, or which penetrate the capsule to discharge into the chamber, are best demonstrated by vertical sections through several different portions of the chamber. Figures 2, 3, and 10 show representative features of the anterior vestibular, the middle respiratory, and the posterior olfactory portions of each species as seen in vertical section.

The vestibular area (Figs. 2, 3) in all three forms is lined in its foremost part with the cornified squamous epithelium of the outer nostrils, which continues inside for a bit and then merges into stratified squamous. This latter type joins the inner or mucosal lining at varying distances inside the nostril, extending for a short space into the ducts of the lateral nasal and lacrimal glands and lining the principal deflecting surfaces of the atrium. At the mucocutaneous junction, beginning anteriorly in the airstream surfaces proper, the typical ciliated respiratory epithelium takes over and lines the rest of the vestibule. Each animal has an effective baffle system at the entry of the vestibule which deflects a large volume of both air and particles away from the inhaled airstream, which is then channeled into the part of the atrium lined with vascular, ciliated, mucous epithelium. The two mammals have also a small external moat just at the outside of the floor of the vestibule which acts as a fluid reservoir receiving and partially retaining fluids from both the lateral nasal and lacrimal gland ducts. A comparison between the single duct mouth of the chicken and carnivore lateral gland system, and the multiducted rodent system is shown in low power microphotographs in Figure 2. It is obvious that the same general result has been obtained by the tubulo-alveolar glands of the carnivore and the multiple ductules from the lateral nasal gland group of the rodent.

All three of these quite divergent forms have a large lateral nasal gland system. The gland and its development and its known functions will be discussed in the review section, but we want to emphasize its significant size

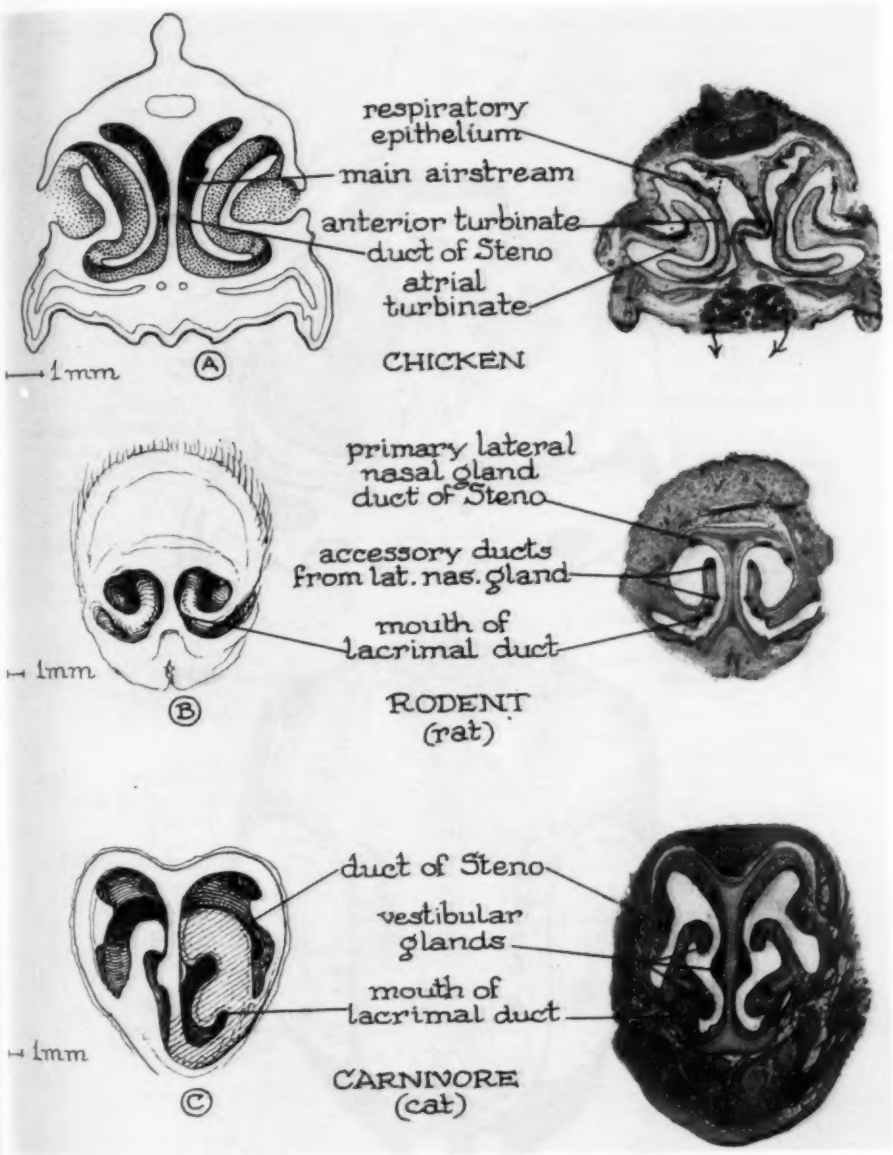


FIG. 2. Vestibular area of chicken (A), rat (B), and cat (C). Left: drawings of anterior part of nasal chambers viewed head-on; chicken beak cut through nostril; tip of nose cut at two levels on cat; rat intact. Right: low power microphotographs of same specimens, cut at levels equivalent to Roman numerals I, Fig. 1.

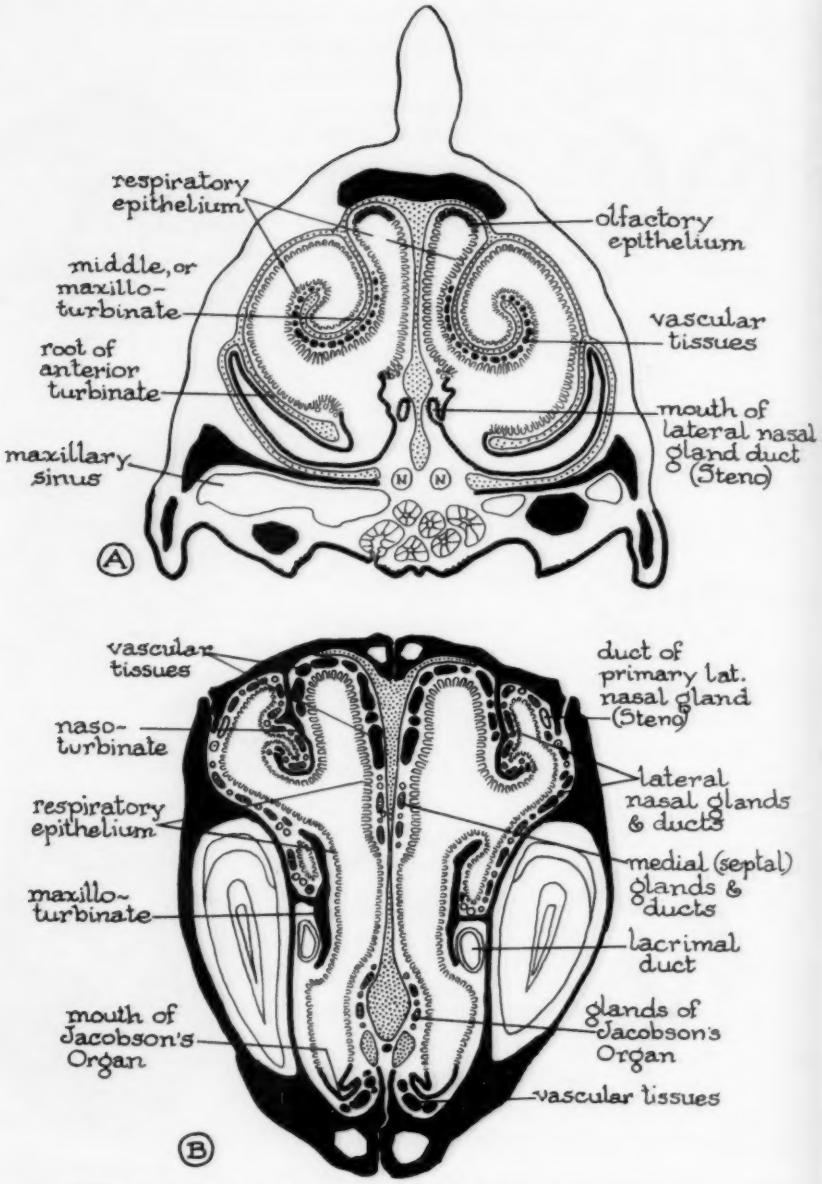


FIG. 3. Diagrams or maps of sections through the anterior part of the respiratory portion of the nasal fossa of a chicken (A) and a rat (B) cut at levels of Roman numerals II, Fig. 1. Cartilage stippled, bone solid black, cornified squamous epithelium solid black line.

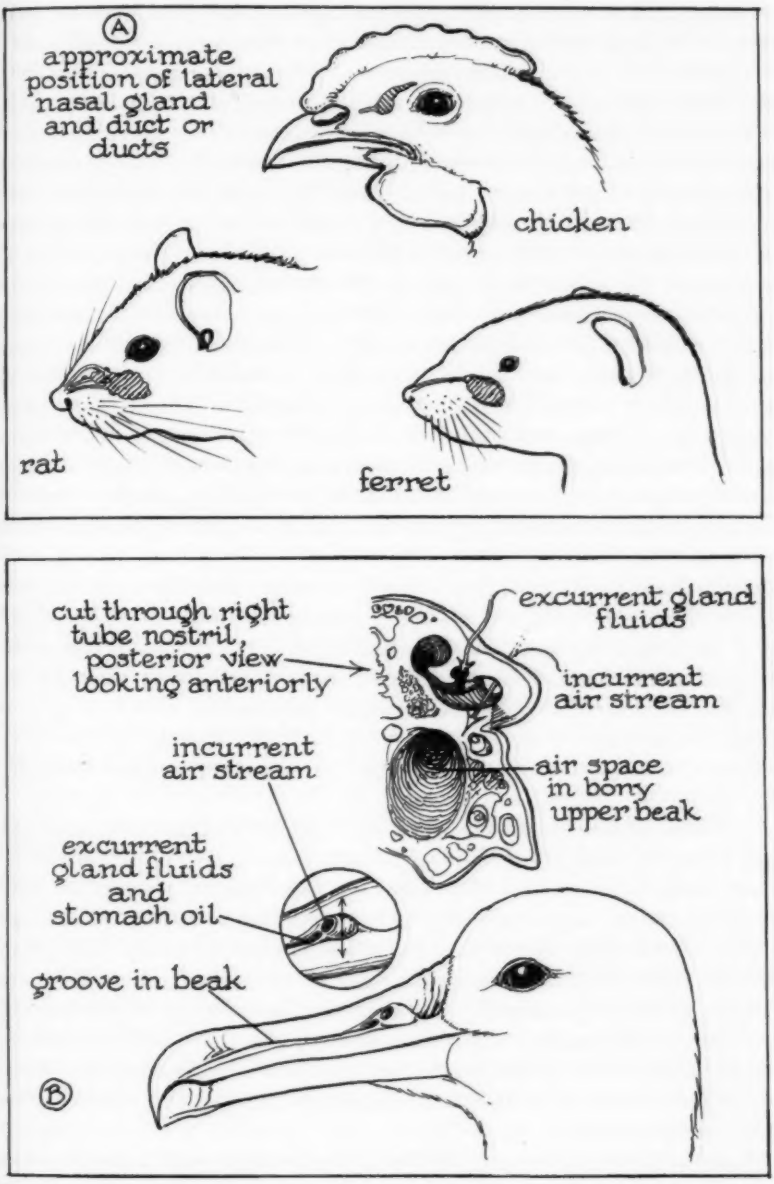


FIG. 4. (A) nasal glands projected on outlines of chicken, rat, and ferret heads. Not to scale. (B) shows tube nostril of Blackfooted Albatross, *Diomedea nigripes*.

and similar exit place in these distantly related animal types. Since the gland is known to assist the kidney in the excretion of excess sodium chloride in water birds (42), the possibility of its role in maintaining water balance in other vertebrates as well is patently open to investigation; the duct mouths are strategically placed not only to excrete waste products conveniently but to utilize the salt water in the necessary process of dissolving olfactory chemicals for stimulation of the sensory fibrils. Figure 4A shows the approximate size and location of the gland projected on the profile views of the three animals; it is within the bony nasal capsule in the two mammals and very slightly encroaches on the supraorbital ridge in the chicken. This gland can become hyperactive as the result of several kinds of stimuli, chemical (82), infectious (15, 16), emotional (47), and mechanical (24), in which case it floods and flushes the atrium. Since the lacrimal fluids can also be stimulated to hyperactivity, and since ciliary currents in this region are directed toward the nostrils, the vestibular part of the nasal chamber in each of the three types is well defended by both structural and fluid resources against the invasion of foreign materials. The normal physiological properties of these several fluid resources have not been fully identified, nor have their possible antibiotic and enzymatic reactions to infectious agents been defined.

Since pelagic birds, according to fossil evidence, have changed little since prehistoric times (14, 48) and since they have special problems in the double use of the nostril for breathing and smelling, it is interesting to look rather closely at the tube nostril of an albatross (this specimen is *Diomedea nigripes*, from Midway Island) to see how ingeniously the cornified tube is designed to withstand the impact of diving and still to effectively separate the channels of the incurrent airstream and the excurrent lateral nasal gland secretions (Fig. 4B).

The middle section, commonly called the respiratory portion, of each of the three animals is liberally provided with mechanisms that could serve defensive as well as normal functions. The mucous-ciliary blanket presumably moves toward the choana and so into the pharynx, sweeping away trapped dusts and particles. Goblet cells secrete into the whole respiratory surface area of the turbinals, which is greatly increased by scrolling or branching of the free lamellae. The surfaces which line the main airstreams have a deeper epithelial layer and a richer vascular supply than do the quieter inner surfaces. The free surfaces of the maxilloturbinals have trigeminal free nerve endings (1, 7) which probably react to irritants, inducing sneezing and flushing, and which may also effect rejection behavior.

The maxillary sinus, or recess, as the case may be, is quite a large organ in all three animals, and in the two mammals is rather complex. Since its opening is in the posterior part of the respiratory section in the rat and ferret, and is

nearly against the cribriform plate in the chicken, we will consider the former first. In both of these, as well as in the cat, dog, and rabbit specimens we have examined, the maxillary recess is in open communication with the nasal fossa and is in fact simply a lateral expansion of the fossa which has hollowed itself out around a big turbinal; the lamellae of the turbinal almost entirely fill the opening and the base divides the recess into an upper and a lower chamber (Fig. 6). Most of the upper chamber and the stem of the turbinal are lined, beneath the ciliated surface, with a gland which varies in depth among the species but which in all of the above-mentioned types empties into the recess by many ductules. It lies in juxtaposition to, and may interdigitate with, the lateral nasal gland body for a part of its length (Figs. 5B, 6B), and the duct of the latter may run through the body of the maxillary gland (Fig. 5C), but there is no functional relation between the two. The maxillary gland appears to be a mass of compound tubulo-alveolar units. Since it may play a role in bacteriostatis (see part II p. 135) it will be of interest to know the ciliary pathways within the recesses of the several species. Hilding (63) followed the approximate ciliary flow in the rabbit sinus) for this may furnish a further clue to its function. As Broman (65) and others have pointed out, the gland could affect the olfactory sensory area. One of our specimens of human foetus of about the seventh month shows anlagen of many groups of glands scattered in the lateral nasal wall. Some of these (Fig. 7B) discharge into the maxillary sinus. They may, of course, be anlagen of the common mucous glands of the adult, but their similarity in location and appearance to the maxillary gland in a ¾ term ferret embryo (Fig. 7A) is striking. The lower or posterior chamber of the dog maxillary recess is the site described by Bast (84) as the principal area containing special methylene blue-staining cells. We have been unable to find reference to any follow-up study of the two types of "special sensory" cells which he described in his paper of 1924 on the maxillary sinus of the dog.

The big maxillary (infraorbital) sinus of the chicken (Fig. 5A) seems to be lined mostly with typical ciliated sinus epithelium, although the cells lining the angle of the medial wall and floor are somewhat deeper. As the cavity approaches the long thin neck leading to the ostium, some quite substantial ciliated cells line the medial wall as well as the dorsolateral angle under the orbit, just at the entry to the neck. Gland activity, then, increases at the mouth of the sinus. Whether there is in fact a compound gland just at this location needs further study; Figure 11 shows a gland structure which may be an accident of sectioning in which a group of single glands was pushed together. The long neck leading to the ostium is again very thin-walled, and the ostium itself is lined with very tall ciliated cells that become mingled at the cribriform face of the ostium with masses of deeply layered clear-staining glands that discharge into the posterior part of the fossa at the choanal brim. The communication

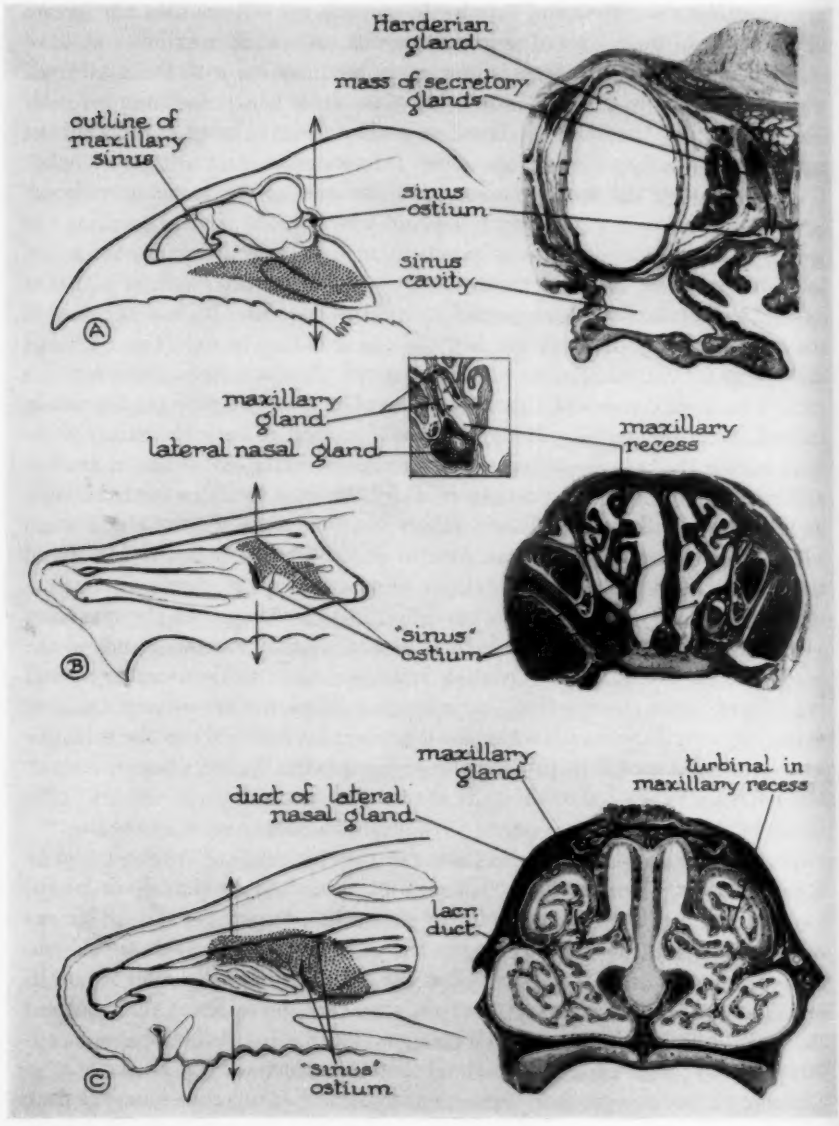


FIG. 5. Left: diagrams of location and extent of maxillary sinus or recess of chicken (A) rat (B) and ferret (C). Right; lowpower microphotographs of sections, cut at levels of arrow-pointed vertical lines on diagrams. Inset shows relationship of lateral nasal and maxillary glands of rat at this level, small arrows indicating directions of duct openings of maxillary glands.

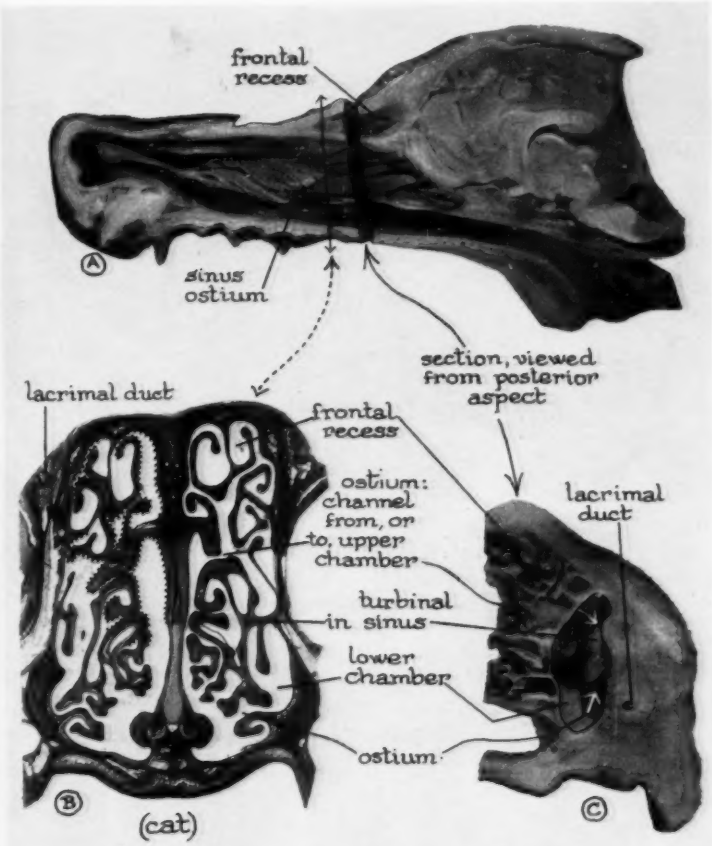


FIG. 6. Maxillary recess area of dog (A), (C), and cat (B). (A) shows medial aspect of right nasal chamber of young beagle, septum removed. (B) is a lowpower microphotograph of an equivalent area in a young cat: on the left side of the section the sensory epithelium is indicated by dotted lines, the maxillary gland by broken lines, and the lateral nasal gland is outlined. The "swell body" and Jacobson's cartilages show clearly at the base of the septum.

between the acellular space inside the posterior concha and the sinus cavity is also shown in Figure 10; the airspace may be subdivided in occasional specimens. The function of the space is not known. The olfactory concha in the phylogenetically ancient penguin contains no airspace; the whole olfactory chamber is even more sequestered than that of the chicken, and the ostium of the maxillary sinus is geographically closely related to the concha (Fig. 8).

The great changes in all of the lining tissues—including the olfactory epi-

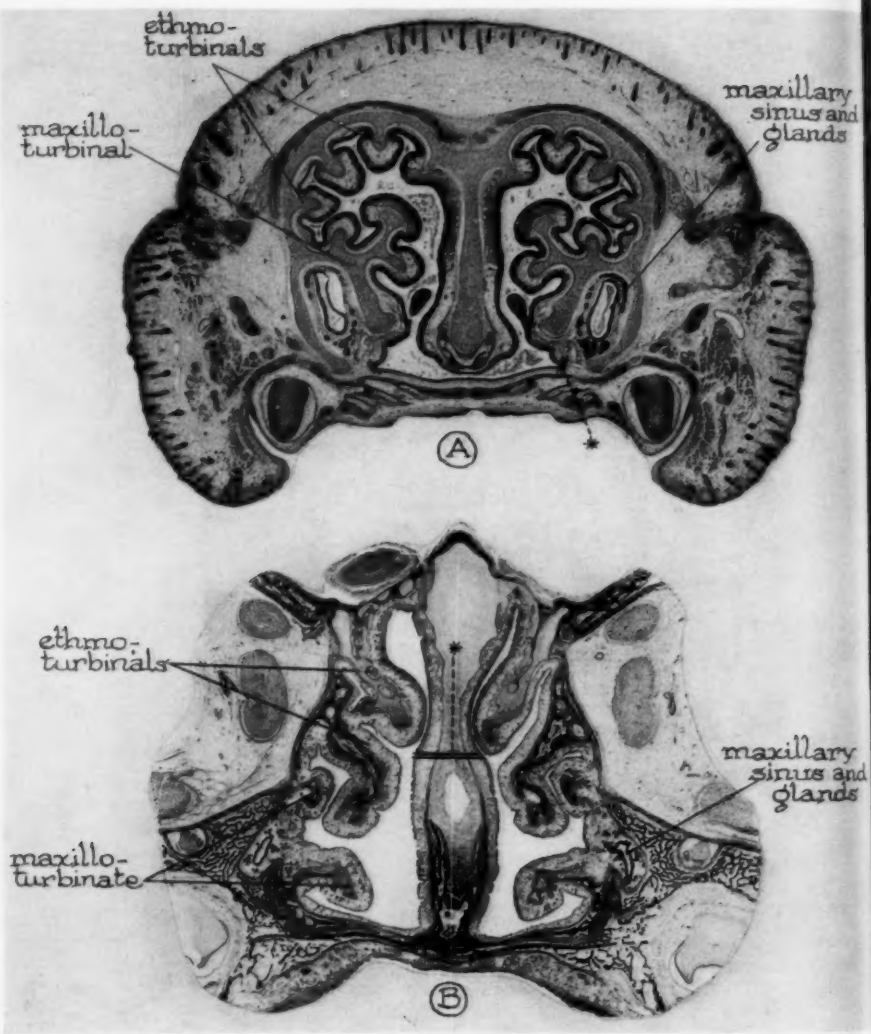


FIG. 7. Low power microphotographs of sections through maxillary sinus areas of embryo ferret (A) and human (B), both about ¾ term. * Indicates border of olfactory epithelium, which lines almost the entire ferret fossa at this level.

thelium, Bowman's glands, the ciliated epithelium of the fossa and of the lacrimal duct, the lateral nasal gland, and the maxillary sinus—produced by coccobacilliform infection in the chicken are shown in Figure 11B in a 4½-week-old bird which showed symptoms of nasal discharge 6 days after intra-

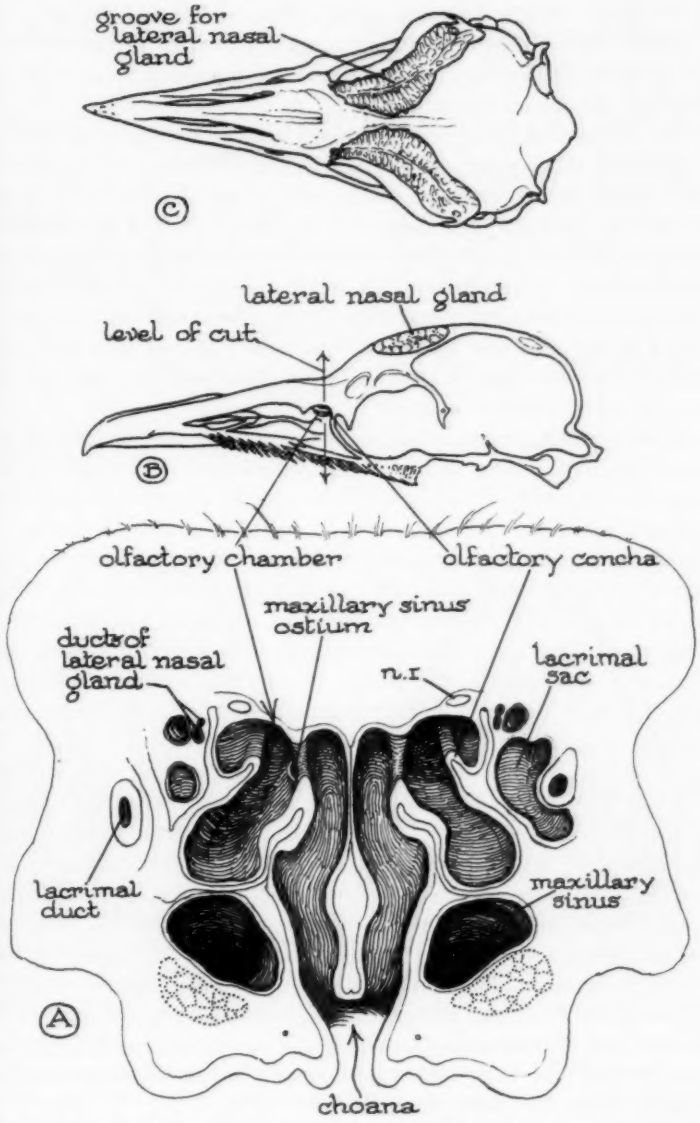


FIG. 8. (A) Drawing of cross section through olfactory area of nasal fossa of a Humboldt's penguin *Spheniscus humboldti*. (B) Sagittal section showing level of cut of (A). (C) Dorsal view of skull of Adelie penguin (*Pygoscelis adēlie*) showing large supraorbital groove for lateral nasal gland.

nasal inoculation with fresh nasal exudate from an infected chicken and was sacrificed at 8 days. In the more chronic stage of this same disease the olfactory mucosa looks somewhat more normal, but the maxillary sinus is as deeply thickened as the respiratory area.

The middle portion of the chicken nasal chamber, unlike the rat and ferret but rather like the human, receives also the fluids from the lacrimal duct. Lacrimal fluids, in other words, in the case of the chicken would flush the respiratory area and go posteriorly to be swallowed and in the case of most mammals would flush the vestibule and spill out the nostrils. The large paraorbital glands that secrete these fluids will be discussed comparatively in the review section; we wish here only to point to a basic difference between the avian and mammalian host that is architectural but that may be of physiological significance as well in tissue adaptations to defense, and thus to the course of upper respiratory disease.

The accessory olfactory vomeronasal organ, or organ of Jacobson, is absent in adult birds as well as in adult primates, although it is fleetingly present in both cases in very early embryos (1). It is highly developed, however, in the keen-scented mammals, and its opening into the nasal cavity is in the middle respiratory area although its position varies from quite far anterior, and apparently unrelated to the nasopalatine passage, to rather central and directly associated with the nasopalatine passage (Figs. 1 and 9). This blind olfactory chamber, with its curiously deep sensory cells arranged with almost mechanical regularity and lacking the glands of Bowman which accompany the olfactory organ in the main chamber, seems to serve a straightforward olfactory purpose, and yet not quite so simply stated a role as is usually attributed to it in the familiar texts. True, in those animals in which there is a direct oronasal channel—the rabbit and ferret are good examples—it may serve principally for oral olfaction of foodstuffs. But in the rodents the opening is very far forward of the nasopalatine canal and seems on an anatomical basis to serve the ventral and lateral airstreams in complement to the main olfactory organ which comes also far forward on the dorsomedial surfaces of the chamber. This would assure a concerted instant stimulation of the sensory mechanism just inside the main chamber. Since the organ of Jacobson has also been shown to be able to suck fluids into and expel them from its cavity (65, 66), and since again a complete understanding of the cellular components of the organ is yet to come, it adds one more open question to the functional and structural constitution of the vertebrate nasal chamber.

The posterior, olfactory portions of the nasal chambers of the three types (Fig. 10) are well protected against drying and injury, set off as they are by a screening system anteriorly and by the cribriform plate wall posteriorly. Olfactory chemicals, essentially free of dusts and well assured of moisture by

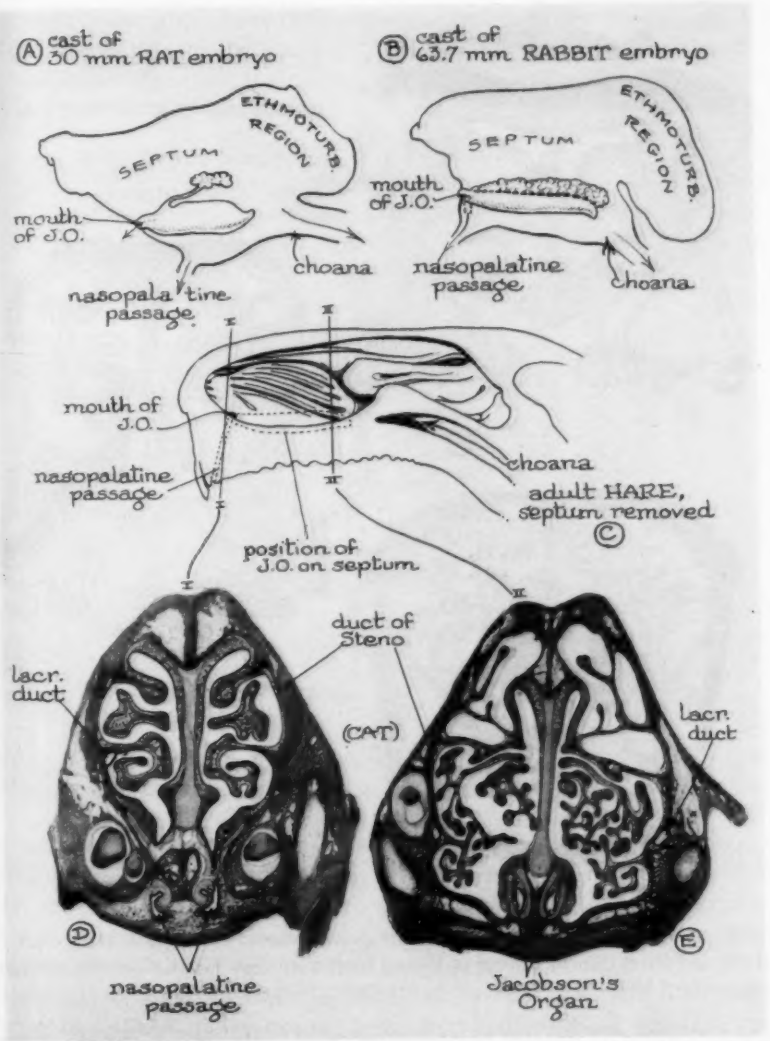


FIG. 9. (A) and (B) diagrams of casts of nasal septa of rat and rabbit embryos, respectively, to show variation in relationships of the mouth of Jacobson's Organ and the naso-palatine passage. (Both after Broman). (C) outline drawing of right nasal cavity of adult Belgian hare showing large maxilloturbinal just distal to vestibule (compare long nose of beagle in Fig. 6 A). (D) and (E) show the naso-palatine passage and Organ of Jacobson respectively in a cat, sectioned at levels equivalent to those indicated on (C). Note strong vomeronasal cartilage around Jacobson's Organ in (E).

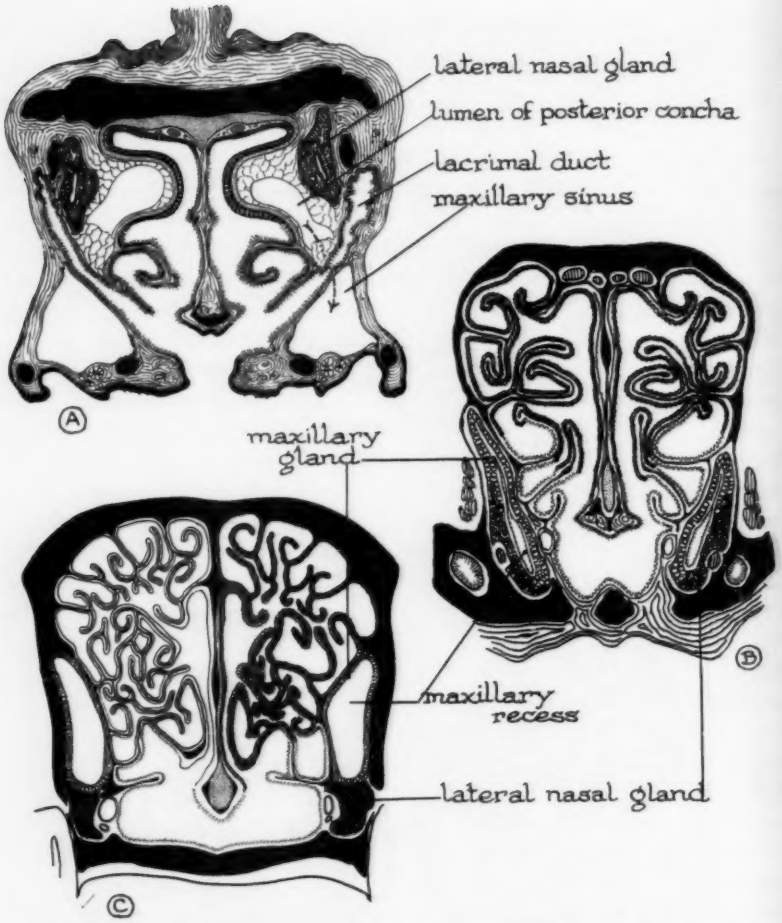
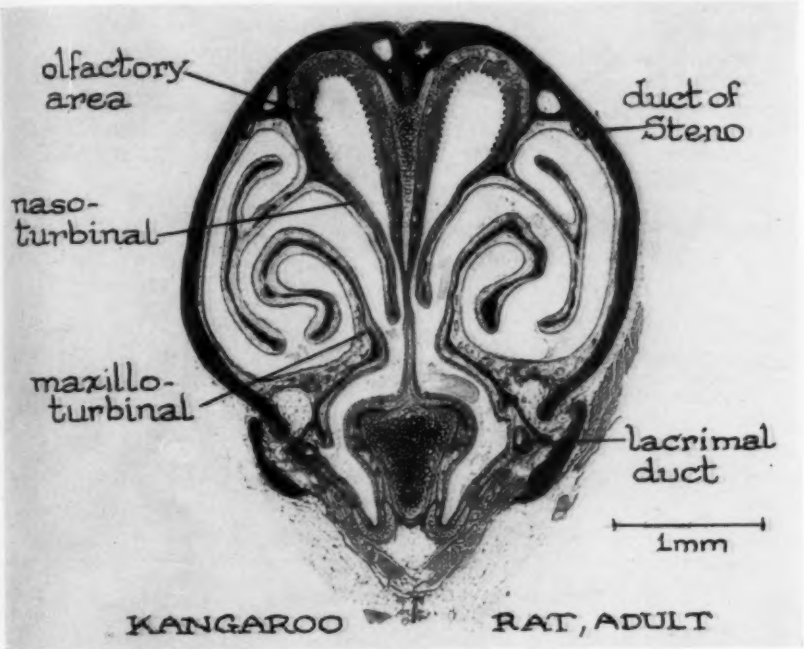


FIG. 10. Diagrams of sections through the posterior nasal chambers of the chicken (A) rat (B) and ferret (C) cut at level of Roman numerals III on Fig. 1. Olfactory epithelium is represented by heavy circles or ovals, respiratory by fine fringed lines.

models the olfactory area is of vital importance to the species, though of some- the complex of gland systems, can then carry out the original purpose of the organ of smell. The primitive olfactory organ of the fish, a blind pouch lined with olfactory mucosa over which sea water flushes rhythmically, was also in contact with the cribriform plate posteriorly and open to the medium anteriorly. In the higher forms, the olfactory organ has not been pushed back, but rather the respiratory equipment of the nose has elongated or otherwise shifted about in meeting structural needs for survival. In each of the laboratory

what lesser importance in the chicken than in the mammals. Its proportional surface, increased as it is by scrolling of the ethmoturbinal lamellae, is clear in the combined sagittal and frontal sections of the individual types. It may be seen in Figure 1B that the sensory mucosa extends far forward in the rat fossa.



The text figure shows the adaptive variation of the fossa in the kangaroo rat (taken at a relative section between cuts I and II of the rat in Figure 1) in which the sensory surface comes even farther forward and is most effectively shielded from the direct impact of airborne dangers. Although the neuroanatomists in particular continue to report new information on the structure of the olfactory sensory system, knowledge of the cellular structure and interrelationships of the components of the olfactory mucous membrane is not yet complete. This specialized epithelium is known to be susceptible of infection (15, 16; Fig. 11) but its defensive, restorative, and regenerative capacities have not been systematically examined either in a single species or in a comparative sense.

The three vertebrate types, then, possess in essence the same basic tissues in the lining mucosae of their upper respiratory tracts. The variations in these intimate tissues in respect to their specialized cells and cell systems, their

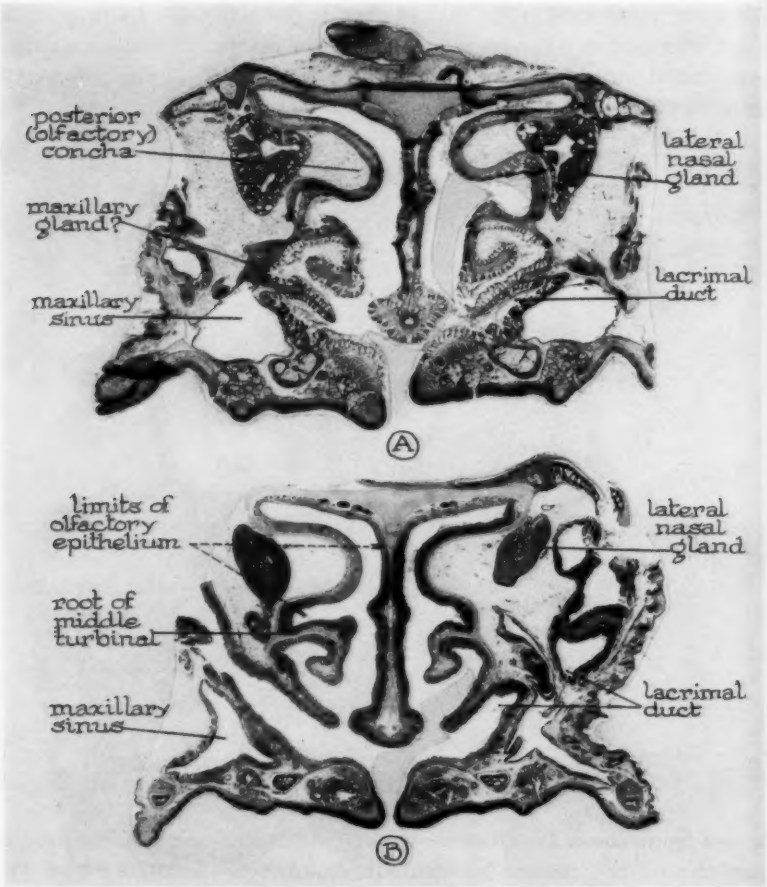


FIG. 11. (A) Low power microphotograph of section through posterior nasal chamber of normal 2 day old chick. Note clear pattern of lateral nasal gland, translucent appearance of epithelial cells, and very delicate sinus epithelium. (B) Section through approximately equivalent portion of fossa of 4½ week-old chicken infected 8 days with a disease clinically resembling cocco-bacilliform coryza. Note changes in lateral nasal gland, all lining epithelia (especially lacrimal duct), and beginning hyperplasia of sinus epithelium.

relative proportions and locations, and the skeletal framework which adapts to their need of support could form a reliable basis upon which to understand the processes of infection in the upper tract. However, until the normal structures are fully understood in regard to their functions, their role in reaction to disease must remain presumptive in direct proportion.

PART II

Adaptive Variation in the Vertebrate Nasal Chamber in Relation to Defense Against Disease

"... it is clearly manifest how diligently the most ingenious Maker of animals contrived lest some filth defile the royal seat, viz. the head. The eyes together with the cavity of the ears had to be moistened, the nose irrigated, the mouth and throat covered with an anointing humor. Yet, neither eyes, nor nose, nor throat will discharge anything if you live agreeably to nature. Indeed, what are tears but signs of a stirred soul? What is the mucus of the nose, or the sputum of the mouth but a token of a life rebelling against the laws of health? ..."

Nicolaus Steno, 1662

1. The olfactory organ of lower vertebrates
   a. Fish
   b. Amphibia
   c. Reptiles
2. The olfactory organ of birds
   a. Anterior and respiratory portions
   b. Olfactory portion
   c. Chicken in experimental disease
3. The olfactory organ of mammals
   a. Turbinates and vomeronasal organ
   b. Compound glands of the nasal cavity
   c. Sinuses and recesses
   d. Other defenses
4. The olfactory sense
5. Regeneration of olfactory mucosa

The morphology, development, and comparative anatomy of the vertebrate olfactory organ have been well documented (1–10). The review that follows is an attempt to relate the adaptive variation of the structures in the nasal chambers of the various species to their possible role in defense against respiratory disease. Comparative features will be considered not from the standpoint of evolution but from that of adaptation, and the phylogenetic framework is used because of the general progression of the organ from a simple to a complex structure. Particular emphasis will be placed on some homologous organs which discharge fluids into the upper tract, with such clues to their adaptive functions as we have been able to find.

Of the three primary vertebrate sense organs, the olfactory is the most consistent in its mode of development and its sensory cell structure (6). Some general features are common to all species. The organ is in fact a highly specialized mucous membrane which contains the receptor terminal fibrils of the

olfactory nerve. It develops as an involution of the ectoderm, retains its relationship to its definitive nerve, and remains in open communication with the medium whether water or air (10, 11, 12). The specialized mucous membrane, the sensory cells, and the nerve itself develop independently of the central nervous system and acquire communication with the olfactory bulb late in embryonic life (13). In contrast to the respiratory epithelium which readily sloughs away if damaged and regenerates steadily under favorable conditions (14, 15), the olfactory epithelium apparently does not regenerate if it is destroyed (see p. 140).

The primitive vertebrate chemoreceptor was probably a concentration of olfactory-type invertebrate receptor cells into a specific area (7); amphioxus has indeed a single cilia pit containing an aggregation of bipolar olfactory cells connected by their axons to the anterior part of the neural tube. In fish the organ is a more or less open tube on the front part of the head and is not functionally associated with the mouth or gills; in all other vertebrates it shares its open channel with the respiratory system. Dual use of the nasal chamber apparently coincided with the land phase of amphibious development, which exposed the joint organ to air drying and airborne particles. The upper respiratory tract of land forms is defined as the area from nostrils to pharynx; respiratory infections may sometimes be contained at this level (16).

The properties of mucus may be subject to individual variation (17), and yet mucus itself has been the companion of animal life since the mystery began. The mucus-ciliary mechanism was introduced into the olfactory organ along with respiratory function. The mucus-secreting cells in the ciliated epithelium are of two types, a deeply placed acinous set and a surface goblet-cell set. When, in infections, the goblet cells desquamate (as they do readily) the deep glands seem to be activated reflexly (17). The physiological defensive properties of mucus have not yet been decisively accounted for, but its rapid changes in viscosity and its obvious stickiness attest its value in mechanical defense, especially in concert with ciliary action.

FISH

It has long been established that fish react to olfactory chemicals. The paired organs of the bony fish are fully exposed rather short open tubes lined with convoluted olfactory mucosa. Parker (7) has reviewed the earlier experiments on various species which showed that fish olfactory organs are used both for orientation and discrimination. Recent laboratory and field experiments with minnows (18) and salmon (19, 20) have demonstrated that choice of the home stream, detection of enemies even from downstream, discrimination between species (21), and rejection behavior are partly or wholly olfactory responses. The hypothesis that some aspects of animal homing behavior are based on olfactory memory and discrimination (22) is thus given considerable impetus. None of the fish, including the cartilaginous and the lung fishes, has

special secretory glands associated with the olfactory organ. It is apparent that disease of this organ would seriously threaten the competitive existence of fish in nature.

AMPHIBIA

The wholesale adjustment of the amphibia to land-living is reflected in the olfactory organ. Since moisture is necessary for both olfaction and respiration, a complex of devices for constant humidity was acquired at the land phase:

1. Sebaceous glands at the junction of outer nose skin and inner mucous membrane;

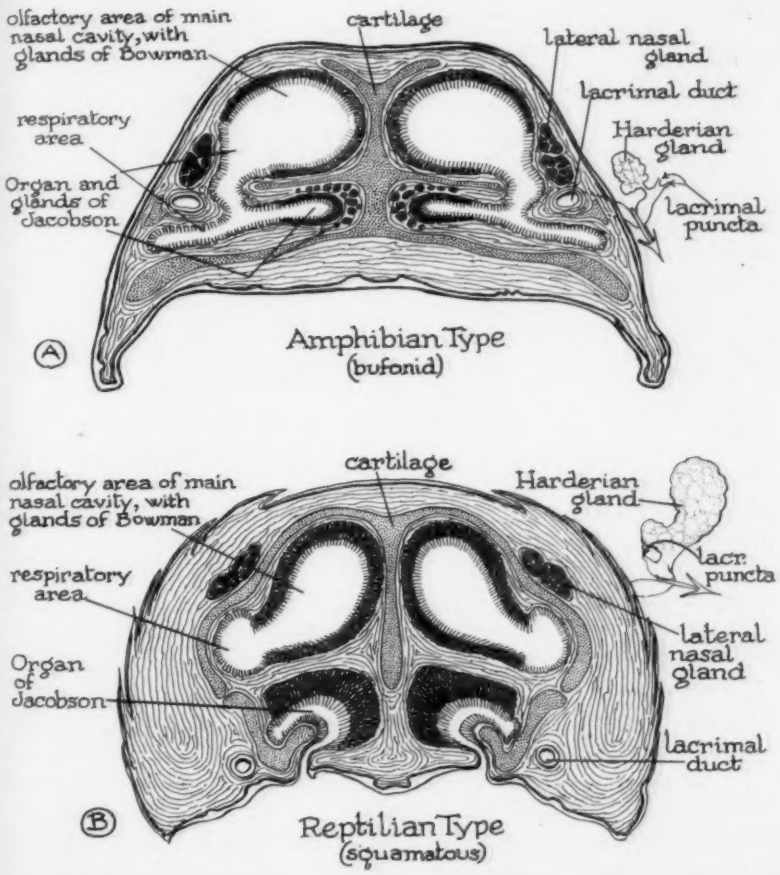


FIG. 12. Diagrammatic sections through approximately the middle of the nasal fossae of composite amphibian and reptilian types. Bones are not indicated, and minor licenses have been taken with antero-posterior locations of secretory organs.

2. Olfactory glands (Bowman's) which specifically lubricate the olfactory cells;

3. The vomeronasal organ, or organ of Jacobson, a diverticulum of olfactory mucosa provided not with olfactory but with its own specific glands. This organ openly communicates with the olfactory organ but is innervated by an additional, vomeronasal, sensory nerve, as well as by the olfactory;

4. Two functionally separate and histologically different glands, the lacrimal and the Harderian, which respectively lubricate the cornea and the nictitating membrane, the secretions of which then enter the common nasolacrimal duct and are transported into the nose;

5. The lateral nasal gland, or gland of Steno, which discharges into the vestibule. These devices are summarized diagrammatically in Figure 12A, except for the sebaceous glands in the outer skin.

Amphibious olfactory reactions have been studied in the spotted newt by Copeland (23). In aquarium tests he found that the initial approach to a moving object was a visual response, but that nosing and snapping reactions were olfactory in stimulus.

One function of the amphibian lateral nasal gland was described by Wilder (24) who found that dirt packed into the nostrils of the lidless newt, *Amphiuma*, was quickly expelled by hypersecretion of the paired gland which, in conjunction with action of the narial muscles, specifically flushed and cleansed the nasal vestibule.

REPTILES

The reptilian nasal chamber has a well defined anterior respiratory and a posterior olfactory section; the posterior part usually contains a true concha or conchal fold which increases the sensory surface. The inter-relationships of the lacrimal-Harderian and vomeronasal organs vary among the species, but Bellairs and Boyd (25a) point out that the reptilian lacrimal apparatus communicates not with the nasal chamber but with the mouth or pharynx and is intimately associated with the large vomeronasal organ (Fig. 12B). Anatomically, the lacrimal duct fluids could act either as accessory salivary organs or as functional resources for the vomeronasal organ (25b, 26).

The vomeronasal organ is more highly developed in snakes and lizards than in any other vertebrates (1). The embryonic connection with the nasal chamber, from which it develops as a lateral pocket in the septal wall, is later occluded so that it communicates only with the mouth while retaining its primary neural connections. This organ is apparently vital for snakes. Wilde (27) in experiments on the garter snake, following a lead by Kahmann (28), established that the forked tongue of the snake is neither tactile nor gustatory but is a carrier of olfactory chemicals directly to the paired vomeronasal organs.

Thus the constant rapid flicking of the tongue from trail to mouth (29, 30). The strike response was also shown to be elicited by olfactory stimulus (27).

Olfaction in lizards was studied by Abel (31) who found that the ofactory organ and the vomeronasal organ had separate but complementary functions. Each area was investigated separately, each was treated operatively, and vision was excluded by blindfold. The olfactory organ was found to be a distance receptor for prey and predator, the vomeronasal organ a close range (8–10 cm.) and oral receptor. Lizards could be trained to unfamiliar smells, could distinguish very subtle differences in odor and could sense moisture as well as odor by means of their noses. Specific odors determined behavior toward mate or enemy.

The paired lateral nasal gland is found in all reptiles and though it varies in relative size and somewhat in position it consistently discharges into the vestibular area by way of a single duct.

BIRDS

There is great variation in the nasal architecture of birds, but an "average" example such as the domestic chicken (Fig. 1) has three conchae quite similar in development and arrangement to the human. The anterior is essentially a baffle and humidifier, the middle scrolled turbinate is respiratory, and the posterior "hill" is olfactory. All birds lack the vomeronasal organ except in very early embryonic life (1), but have a well developed paraorbital and lateral nasal gland apparatus. The nictitating membrane reaches its greatest development in birds and is apparently especially important to diving birds, in which—as in the frog—it is transparent centrally when stretched across the eye (32). As may be expected, the Harderian gland of aquatic forms is very large; it pours a fatty secretion onto the under surface of the membrane and lubricates the elastic fibers.

The lateral nasal gland is a consistent avian structure (33–37), varying greatly in size and considerably in location. It is significantly enlarged in marine birds, so greatly in fact that in eight of the orders a groove is hollowed in the supraorbital ridge as the bone develops around the gland (35–38) (Fig. 8). Grooves are generally small in freshwater and large in marine forms. Skulls of the Cretaceous Herperornis and Ichthyornis both show large supraorbital grooves (35). The gland itself is bilateral, each unit usually comprising two lobes, each of which in turn has developed from an intrinsic duct, one from a medial septal, one from a lateral. The ducts vary in relative size among the species. Gallinaceous birds have only the lateral duct which curiously leads under the nasal chamber floor to discharge at the site of the mouth of the missing median duct (Fig. 3). The gland is innervated by the ophthalmic branch of the trigeminal nerve and receives its blood supply from the anterior ophthalmic artery.

Over thirty years ago Schiöler (39) noted that the European duck had small lateral nasal glands while the distinctly marine Greenland species had large ones. The salt-water implication was defined further by Heinroth (40) who showed that the nasal glands of eider ducks degenerated if a freshwater diet was substituted for salt water. In experiments with descendants of these same ducks, Schildmacher (41) then showed explicitly that the glands enlarged or degenerated in direct proportion to the salinity of the water diet. Degenerate glands were found to be of good size but functionless, bloodless, lacking a lumen, and possessing a tightly adherent epithelium. Even the bony nasal process was affected, becoming broader as the gland lost function.

In a continuing series of physiological studies of salt excretion, Schmidt-Nielsen and several collaborators (42) have demonstrated that the lateral nasal gland assists the kidney as an excretory organ for excess sodium chloride in marine birds, yielding up to ten times the salt excreted by the kidneys after a feeding of excess salt. Since the penguin used in one of these assays had to our knowledge been on a fresh-water diet for many months since its captivity, even these very significant tests may not represent the full natural excretory potential. Eels are known to excrete salt from the head region (43), the mechanism apparently being secretory cells which react to various concentrations of NaCl (44, 45). Such cells are in the gills of eels and teleosts, increasing in number and in proportion to the mucous cells as young fish increase in size (46). Since no physiological studies directly concerned with the amphibian lateral nasal glands have apparently been reported, it is not clear whether these cells may have adapted to amphibian excretory function and in turn to the primitive avian salt excretory system.

Frings (47b) has found the nasal gland to be affected by excitement in both the black-footed and Laysan albatross, which drip rapidly from the nostrils when agitated. The dripping of nasal gland fluids seems to have no direct relation to the discharge of stomach oil through the nostrils. The interesting habit (of the fulmar in particular and of other petrels and some albatrosses) of squirting oil through the nostrils and vomiting it from the mouth seems to stem from various kinds of stimuli and to represent particular behavior (48, 49a, b). The oil is secreted in the stomach and may be stored in the proventriculus (50a, b). It may then be forcibly ejected from the mouth for a distance of some feet as an act of defense, after which residual oil may drip from the nostrils. Or it may be exuded from the nostrils into the beak grooves (see Fig. 4B) to be used in preening in the same way that oil from the preen gland (which it resembles chemically (51)) is used. Or it may be fed to a mate during courting behavior, or to a young nestling, which would benefit from its rich vitamin A content. The waxy oil is highly colored and foul smelling, the odor being both pervasive and persistent, so that it would be an excellent device for marking

territory, nest, and family for proximal olfactory reception and terminal homing. It is relished as food by the Maoris (48, 52) and has been reported to be effective in treating bronchitis (52), though principally by way of contributing to a gain in weight. The design of the tube nostrils of our specimen of albatross (*Diomedea nigripes*) specifically segregates the efferent lateral duct fluid from the respiratory airstreams (Fig. 4B). Whether the double gland and discrete ducts are related to separate functions is not known. Multiple functions of other compound glands are well recognized (53).

Though birds are designated as microsmatic, or feeble-scented, in most text books, there are notable exceptions to this generalization. Both W. K. Parker (54) and T. J. Parker (55) pointed out the extensive olfactory apparatus in Apteryx; Matthes (1) proposed that ground-living birds such as partridges might be expected to have keen scent, and indeed Frings (49 a) found a significant chemo-receptivity in the bob-white quail. Burne (56) noted the very large olfactory brain of the wandering albatross. Benham (57), impressed by the size of Apteryx olfactory conchae, had some simple food-finding tests done on a captive roa-roa and found that this small relative of the kiwi could precisely extract bits of meat hidden under four inches of packed earth without disturbing the surrounding surface. He incidentally noted the continual snuffling of these night birds as they searched, reminiscent of mammalian spoor-sniffing.

Strong (58) noted both the large olfactory lobes and big trigeminal (ophthalmic branch) nerve in marine birds. He pointed out, as have others, that the olfactory organs of the so-called lower bird groups are large, and seem to become progressively smaller in the "higher" forms.

Murphy (48) described albatross behavior that showed keen olfactory discrimination under circumstances that ruled out vision as the perceptive factor. The aquatic birds we have examined (penguin, Canada goose, tern, shearwater, and albatross) have extensive olfactory chambers. The penguin olfactory area looks small in a sagittal section, but shows as a capacious chamber when viewed in frontal section (Fig. 8). This region appears to be specifically served by the maxillary sinus which has its ostium atypically on the medial wall. We have been unable to find reports of the pathology of the avian nasal gland; it was mentioned as one of the tissues in which foci of infection were found in experimental Newcastle disease virus of chicks (16) but there was no connotation of specific pathology of the gland; initial destruction of the mucous membranes by this virus was uneven. When minimal volumes of inocula were given intranasally the resultant infection was limited to the upper respiratory tract.

Architecturally, the chicken nasal chamber is a good model for human-oriented experiments, especially on such environmental factors as dusts, humidity, and temperature, because it has three simple conchae, a maxillary sinus, and a centrally located lacrimal duct opening which may be directed

either toward the pharynx or the nares. Lowry (59) has recently shown that Newcastle disease virus inoculated into the nares of 4-day-old chicks spread to trachea and lungs more extensively in a hot dry than in a hot wet environment; more in a cold wet than in a hot wet; and to an essentially equal degree in cold wet and cold dry environments. The infection spread from turbinates to lungs in about 48 hours under favorable conditions. Tracheal ciliary activity was slower under hot dry than hot wet conditions, and tissue damage was more marked in the dry heat; he suggested that the breakdown of the muco-ciliary defenses was the factor accounting for the spread of infections in the hot dry environment.

There is a coccobacilliform agent which produces chronic, non-fatal contagious upper respiratory disease in chickens (60a). A similar coccobacilliform agent causes a different series of reactions in mice (transient early rhinitis and subsequent pneumonia and otitis media) (60b); a similar agent again causes infectious catarrh, resembling the chicken disease, in albino rats (60c). In the chicken disease the lungs and middle ear are not affected, but there is extensive inflammation of the nasal passages, discharge for several weeks, and eventual symptomatic recovery. A chronic, non-fatal respiratory disease resembling Nelson's coccobacilliform coryza is annually epidemic in pens of young chickens at East Suffolk in England. Studies of this infection done at the Veterinary Laboratory in Weybridge, England, have shown that (61) there are symptoms first of clear viscid nasal discharge, then facial (maxillary sinus) swelling and lacrimation. As may be seen in low-power microphotographs of this disease there is extensive hyperplasia of the lining mucosae (Fig. 11).

MAMMALS

The intranasal structures are most highly developed in relative size and complexity in the mammals. We will review only those which seem to have a defensive role: principally the conchae, the compound glands, and the vomeronasal organ. This latter structure is present in all mammalian embryos and varies in adult forms from a prominent to an atrophied organ (87d).

Whatever else they may contribute toward warming and moistening the nasal passages, the maxilloturbinals of some mammals have a definite defensive function. This was demonstrated in tests concerned with filtration of silica dusts. Schiller and Worth (62) found that in short-nosed dogs roughly 35 per cent of particles under 2μ were filtered out by these conchae; in long-nosed dogs (Fig. 6A) about 50–70 per cent, and in rabbits, which have quite complex maxilloturbinals (Fig. 9C), about 86 per cent. The maxillary concha contains free trigeminal nerve fibers (1, 7), as does the whole nasal mucous lining, so that inspired irritants would instantly stimulate this threshold area.

Hilding (63b) showed clearly, in experiments on dogs and rabbits, that if

one nostril was surgically closed, intranasal columnar epithelium was altered in proportion to the degree of exposure to the impact of cold air. Change was most marked in areas which are normally most exposed (endoturbinal surfaces, septum). The turbinate of both species hypertrophied and the mucosa of the dog frontal recess area was markedly altered, becoming thicker and more glandular. In the most sheltered areas, cuboidal cells became tall columnar cells.

The posterior conchae, or ethmoturbinals, are so constructed that the broad concavity of sensory surface receives the initial impact of inspired particles; in keen-scented animals this surface is tremendously increased by intricate branching (Figs. 6, 10). Allison (64) has estimated 50,000,000 olfactory cells in each paired organ in the rabbit. The olfactory conchae of the less keen-scented mammals—primates, platypus, whales, and pinnipedes—are simple or vestigial.

The vomeronasal organ is also well developed in the keen-scented species. In the rabbit Allison (6) found approximately 1,650,000 receptor cells per side in this organ, or about $\frac{1}{30}$ the total olfactory sense equipment. The organ was shown rather crudely by Broman (65a) in the horse, and then definitively by Hamlin (66) in the rabbit to be able to suck fluids into its cavity and expel them by action of the erectile tissues in the surrounding cavernous spaces. Cartilaginous support prevents collapse of the organ. This capacity would be of value in flushing exhausted receptor cells as well as in providing a fluid reservoir against an emergency of desiccation. The antibiotic potential of the organ, its glands, and its flushing mechanism is not known.

The lacrimal, Harderian, lateral nasal, and maxillary glands are prominent structures in keen-scented mammals. The somewhat variable lacrimal glands and the gland of Harder again secondarily discharge secretions into the nasal chamber by way of the common nasolacrimal duct, usually into the vestibular area.

The nictitating membrane has two quite separate glands (67), a fact that has caused some confusion. The well-known Harderian gland of mixed structure secretes an oily material which discharges onto the conjunctiva of the nictitating membrane (68) by way of ducts through the cartilage (69). The lesser known nictitating membrane gland is apparently associated with and partly embedded in the cartilage and opens onto the floor of the membrane sac; it is lacking in some major species of rodent. The Harderian gland is rarely lacking except in the primates. The function of the famous porphyrin content of the Harderian gland (70) which produces "tears of blood" in some rodents has not been entirely accounted for, though Figge (71) has found porphyrin enzyme production to be related to puberty in both mice and humans. The gland is generally rudimentary in animals having a rudimentary nictitating membrane; it is atypical, small and granular in the horse, large in the sheep and ox (68).

Not often alluded to, but of great importance in some of the ungulates are

glands which open onto the conjunctival bulb by duct mouths scattered over the bulb surface (67). In swine, mucous glands discharge in this way, and in cattle true sweat glands do so. Shope (72) has observed that the lids of pigs infected with hog cholera are often stuck together; he does not confirm the statement (68) that the lids become adherent with mucus in swine influenza. Since the aquatic dugong has neither Harderian nor lacrimal glands (73), yet it is known to produce copious "dugong's tears" (74), it may be that these animals, like the cetaceae (67) compensate with intrinsic conjunctival glands.

Lacrimal glands are fairly constant in land mammals and are lacking in pinnipedes, cetaceans, hippopotami and some other largely aquatic forms. Curiously, they are absent in elephants and peccaries (67). The lacrimal gland generally consists of two subdivisions which may be multiducted and indifferently segregated, as in the human, or widely separated into internal and external orbital parts having distinct but proximally converging ducts as in most rodents and many carnivores. The rodent external part has been mistaken for a salivary gland because of its deviation into the parotid area; this external gland is known to have uncommon mitotic (75) and physiological (76) properties but neither its normal nor pathological functions have been defined. Sebruyns (77) found rabbit, cat and ox lacrimal glands to be tubo-acinous and muco-serous, with a preponderance of serous cells; calciform cells were seen in the lobar canal of the lacrimal gland in the cat and rabbit.

A gland apparatus that is not functionally but is often anatomically associated with the orbit is the pre-orbital gland in the "lacrimal pit" of ruminants. A distinctive characteristic of the deer family, this pit is a long invagination or sac formed in the integument and lined with an abundance of sudoriporous or apocrine-type glands (73) which seem to serve in substitution of the periproctal scent glands which these forms lack. Hediger (78) notes that lacrimal pit gland secretion is rubbed on trees by male antelopes to establish territory. Facial apocrine and holocrine glands have also been described in bats (79). Whatever the structural variations in the orbital gland systems may signify, it is evident that no simple or single environmental factor is the principal adaptive effector.

The lateral nasal gland, after the fetal stage, is in most mammals a compound gland with a single efferent duct (of Steno) opening into the vestibule. In the equine family it is reduced to a small granular mass in the wall of the maxillary sinus, though the duct, after following an atypical course, eventually discharges into the vestibule (80). Adult bovines lack the gland entirely. Rodents and rabbits have a network of accessory ducts in addition to the primary duct of Steno, each draining its particular portion of the lateral nasal gland group (65b) (Fig. 4A), but they all discharge into the vestibule. Information on the function and histology of the lateral nasal gland of mammals is scanty. A

clue to one function was discovered by Trautmann (81) and Gylek (82) within a year of one another; although these two authors had some differences of opinion, both demonstrated that the lateral nasal glands of the dog were a major source of vestibular fluid and (82) that pilocarpine stimulated it to hypersecrete. Bast's histological observations on this gland will be included below with those of the maxillary gland.

The maxillary sinus in some species may have a multiple function. Our observations on the compound gland secreting into the maxillary recess of the dog, cat, rat, and ferret, called attention to the significant size of this organ and to a possible homologue in the human embryo (115 part I). Linton (83) studied the comparative antibacterial action of secretions of the maxillary sinuses of rabbits and the frontal sinuses of dogs. He demonstrated that secretions of the maxillary sinus failed to support growth of a lysozyme-soluble coccus from the air, the bacterial count showing a sharp initial drop and a subsequent progressive decrease. The frontal sinus secretions on the other hand supported a progressive increase in bacterial growth. The possible role of the large maxillary gland in bacteriostasis, and the lack of such a gland in the frontal recess cannot be overlooked.

Bast (84) described two types of cells in the dog maxillary recess which he called olfactory and "special sensory," respectively. He has also furnished the only comparative data on the histology of the maxillary and lateral nasal glands that we have been able to find. He described the lateral nasal gland as a single compound tubulo-alveolar mass collecting ultimately into a single duct, and the maxillary groups of glands as opening into the sinus by many individual ducts (Figs. 5, 6). Granules of the two gland types showed different staining properties, refractive indices, and chemical properties, and the mitochondria were different morphologically. The only reference we have found to the cellular pathology of the lateral nasal gland in mammals is of its transient hyperactivity in experimental influenza infection in the ferret (15), an animal in which it is well developed (Fig. 5C).

Broman (65b) suggested that, in rodents at any rate, the primary function of the maxillary gland secretions is irrigation of the olfactory mucosa. The relationships between the ostia of the sinuses, or openings of the recesses as the case may be, and the olfactory organ of the chicken, penguin, and several small laboratory animals were illustrated in the preceding section.

Since there is obviously a need to distinguish between a sinus and a recess if such structures are homologous, and since sinusitis is a well-known companion of clinical upper tract disease, we will consider very briefly some of the principles underlying their variations in some of the orders. The comparative anatomy of mammalian sinuses has been described by Paulli (85a) and reviewed from different points of view by Matthes (1), Nemours (86) and Negus

(87b) among others; anthropoid sinuses specifically by Cave (88); and human by Schaeffer (5) and Proetz (89). Paulli designates a sinus as an extension of nasal mucosa into the bony skull in such a way that it becomes a cavity or air space completely divorced from the nasal fossa and communicating with it only through a sharply demarcated opening, or ostium, which represents the site of developmental outpouching from the nasal cavity (85a). This incursion into bone originally develops in the same way that the turbinates do, by successive evaginations of the lateral walls into meatuses which subdivide in turn and carve the lamellae and roots of the turbinals as they push further into the bones. In some cases a meatus will in turn begin to burrow still deeper into the bone, swelling as it pushes, somewhat in the manner of an inflating balloon; in this way an air sinus is formed, the original site of evagination remaining as a small ostium.

The difference between a frontal or maxillary sinus and a frontal or maxillary recess is one of degree of pneumatization and of the relative size of the channel of communication. The maxillary recess of a dog or cat communicates with the fossa by an opening that extends from the roof of the fossa to the floor, and the same may be said of the frontal and sphenoidal excavations. The maxillary sinus of the chicken, penguin, and human, however, is a sizable air space communicating very narrowly with the nasal fossa. Since these excavations develop in the same way and encroach on the same bones (principally) they are homologous, and are called sinuses in the small mammals by tradition.

As far as the extensive pneumatization of the skulls of some of the large ungulates is concerned, Paulli (85a, b, c) points out that this has little phylogenetic importance because: 1) the whole ethmoidal system has tremendous adaptability; 2) the air spaces may occupy analogous positions in two species yet arise from different parts of the nasal chamber (an example is the cranial position of the maxillary sinus of the giraffe which occupies exactly the same relative position as does the ethmoidal complex in the cow); 3) there may be great difference even between the two sides of one skull, and variation within a single species is enormous. The ethmoidal cells and true frontal sinus are peculiar to the two African anthropoids and man (85a), those of man being much more variable in form and in site of outgrowth.

Boas and Paulli (90) point out that in young animals the brain is relatively large, the teeth and masticating muscles relatively weak, the facial part of the skull small, muscle crests undeveloped, and air sinuses small if present at all. These juvenile peculiarities are then successively wiped out, and the form of the skull is determined by the functions of its organs: teeth, chewing apparatus, organs of vision and smell and sometimes of hearing, and the relative size of the brain parts which serve these organs. The head that contains this equipment must be light enough for the neck muscles to support, and in this respect

the amount of chewing an animal does in relation to its total size affects the pneumatization of its skull. Carnivores gulp, rodents nibble and grind, primates chew cursorily, and herbivores chew and grind most of their waking hours. The large ruminants, therefore, requiring a tooth area of roughly the $\frac{2}{3}$ power of their total weight (91) need not only a huge grinding surface but a powerful frame upon which to attach the muscles needed for the job. If such crests were built onto solid bone, the head of a horse or an elephant would be unmanageably heavy. Therefore the structural engineering of the head acquires strength without weight by means of the girder principle (92). In further support of this thesis, Paulli (85b) points out that the pneumatization of a given animal type of skull is directly proportional to the size of the animal, a large dog, for example, having an extensive ethmoidal system while in a small dog this system is markedly reduced.

In regard to maxillary sinus pathology, some question of ciliary loss, scarring, "ventilation," and drainage in experimental surgery are explored in Hilding's work on the rabbit (63c). He had previously found that stripping bands of mucosa from the concave surface of a sinus resulted in elevated bands of contracted scar tissue. These scars interfered with drainage in proportion to their elevation and if high enough would dam the flow of secretion. Microscopic examination of these scars later showed them to be covered with well-ciliated epithelium, but they nonetheless positively obstructed drainage. If mucosal bands were stripped from flat areas, the scars were not elevated and did not interfere with ciliary flow. He then wanted to see what effect enlargement—"ventilation"—of the ostium would have on the drainage of a normal sinus. Again using rabbits, he enlarged the ostium by cutting windows in the medial wall of the recess at various locations. No matter where such windows were made, suppurative sinusitis resulted, though it was more marked if windows were near the ostium than if they were not. He concluded that the region of the ostium could not with impunity be interfered with surgically. Especially interesting in the light of Linton's previously cited work (83) is the fact that Hilding ruled out infection resulting from surgery as the cause of postoperative sinusitis in these rabbits by opening six control rabbit sinuses without aseptic precautions, using non-sterile instruments. All six healed without infection or disturbance.

A specialized vascular arrangement in the mammalian nose may have defensive significance in relation to delicate and rapid response to temperature variation. Dawes and Prichard (93) investigated the arteriovenous anastomoses in the noses of several domestic animals, principally the dog. They found the most numerous and largest anastomosing vessels adjacent to the surfaces exposed to air streams, and described veins with thick muscular walls that might explain the phenomenon of vascular engorgement and sudden release.

They proposed a sensitive circulatory control in the nasal chamber by means of the glandular and subepithelial capillary beds which would respond, in conjunction with the surface vessels, to changes of temperature and humidity. Chilling of the human external body surface has been shown (94) to cause a lowering of the temperature within the nasal chamber, and in experiments with mice (95) lowering the environmental humidity to 25–30% at 21° C for three weeks was shown to effect a change in the nasal tract bacterial flora.

Two points which have been emphasized and fully discussed in the literature on human anatomy and pathology should not be overlooked as pertinent to experimental disease. These are the strategic position of the nasopharynx and the dependence of the safety of the entire upper tract on the mucus-ciliary mechanism (96, 97). Particles inhaled nasally are principally screened out by the vibrissae, but smaller ones become adherent to the mucus sheet which is swept by the cilia toward the pharynx. The question of the degree of filtration of infectious particles by mechanical means as against the role of lysozyme, antibodies, or other antibiotic constituents of the nasal and nasolacrimal secretions has not been settled. The change in the character of saliva and mucus, the chain of abnormal events set off by cessation of ciliary motion, or by minor anatomical anomalies, the importance of lymphoid tissues in infection and convalescence, are common to all forms.

Added, then, to the activity of the erectile tissues, the tubulo-alveolar glands in the tunica propria, the mucous glands, and the possible transudation of fluids through tissues in the mammalian nose, secretory fluids can be stimulated to discharge hyperactively into the passages from the lateral nasal gland, the maxillary glands, the vomeronasal organ, and secondarily from the two gland types of the orbit. Such reactions are known to be stimulated variously by desiccation, flooding, irritation, alkaloid injection (81), change of temperature, mechanical impact, and chemoreception.

THE OLFACTORY SENSE

Reflected in the defensive properties of the nose is the fact that olfaction may be a vital sense, as in the hedgehog (98), while again it may be discarded in the foetal stage as in the anosmic species of whale. Parker (7) pointed out that vertebrate olfaction has been chiefly concerned with environmental odors. Contemporary land forms depend on smell for distance reception and for proximal discrimination. Some form of saline solution is essential for olfactory reception; rinsing the sensory area with clear water stimulates mucous secretion in such amounts that the receptor fibrils are submerged, and continued flooding can cause permanent injury (7). Rollett (99) found that different olfactory sensations recover at different rates after induced anosmia. Anosmia has been inferred to be related to a lack of pigment cells in albinism (6). Pigment in relation to olfaction has been reviewed by Negus (87c).

Elsberg (100) in human experiments related chiefly to methods of locating olfactory lobe tumors by olfactory tests, developed a widely used method of testing acuity and fatigue and observed that recovery of full sensation following acute upper tract infection in humans may require 8 weeks. The extremely complex neurophysiology of olfactory chemoreception is beyond the scope of this review[4] except to relate its known general principles to the natural habits and defensive adaptations in experimental hosts. An animal reacts to its own odor, to individual differences within its species, and to those of other species within its milieu (9). It reacts to specific food odors both spontaneously and in relation to learning.

Adrian (101a, b) and his collaborators showed that the strength of olfactory stimuli might relate to the pressure of mechanical stimuli rather than to the volume of chemical stimuli. Each inspiration produces a burst of impulses in the olfactory bulb, whether particulate or not; if, however, highly volatile particles, such as xylol or acetone, are received, the bursts regulated by normal breathing may be inundated by continuous discharges. Such hyperactivity would require instant washing of receptor cells. The health of the olfactory and vomeronasal irrigation systems as well as the maintenance of a proper saline balance therefore seem requisite. Paulsen (102) demonstrated that in quiet breathing particles were not carried to the human olfactory area but that sniffing, excited by the slight (mechanical) stimulus initiated by quiet breathing, created eddies which then set off the chemical excitation. Baradi and Bourne (103) have found various phosphatases to be localized in specific cell types in the olfactory mucosa, and present the hypothesis that the chemical reactions involved in this breakdown may cause ionic changes which induce currents in nearby nerve fibers. Inhibitory substances would increase the number of possible combinations of nerve impulse patterns.

The behavioral sciences currently approach the subject of learned responses to olfactory behavior from several directions which are converging under a neurophysiological umbrella. Chiefly they concern conditional responses of physiologic processes (mostly hunger) to olfactory stimuli (104–106), and the olfactory sense in instinctive, social, and homing behavior (see appendix of texts and reviews). The many studies run a highly specialized course from suckling responses in the newborn to terminal navigation in birds, and the prerequisite of healthy olfactory reception is self evident.

A final factor which may influence olfaction is the larynx. Negus (87a) in his text on the larynx suggests that the vertebrate epiglottis is a means of ensuring olfaction while swallowing and whilst the mouth is open, a capacity that is degenerate in man. Extirpation of the larynx apparently interferes with the olfactory mechanism (107); whether, however, this actually disturbs the

[4] Moncrieff (8) reviews the field of olfactory physiology up to 1945; Patton (appendix) covers the years 1945–49.

mechanics of swallowing or simply circumvents the olfactory organ by means of a tracheotomy tube is not entirely clear.

The gradual ascendancy of primate vision and intelligence over olfaction, and of prehensile hands over prehensile snouts in performing the tasks of finding, testing, and gathering food; and the concomitant changes in the shape of the skull as it has accommodated the position of the eyes and growth of the brain has been exhaustively commented upon in comparative literature. In reference to the nasal chamber, Negus (87b) has been interested in the evolution and exotic variation of the nose, and Cave (88) has contributed a careful study of the phylogeny of the primate nasal apparatus which stresses the sinus systems.

REGENERATION OF OLFACTORY MUCOSA

Whether post-embryonic olfactory epithelium can regenerate is not yet fully settled. Evidence seems to weigh against the functional regeneration of the sensory cells. The regeneration of olfactory sensory cells and olfactory brain tissues have been described by experimental embryologists, principally in early amphibian embryos (2). The great body of experimental work on adult animals has centered about lesions or extirpations of olfactory lobes and nerves rather than destruction of the sensory cells. It has been clearly demonstrated that there is a two-way intercourse between the fluid spaces surrounding the central and sensory nerve elements (108, 109) which is of direct concern in mucosal infection.

Reports of experimental regeneration of adult animal olfactory membrane have not been confirmed. Nagahara (110) described regeneration of some olfactory nerve cell fibers 90 days following transection of the olfactory nerve, but the evidence upon which the interpretative drawings were based was not conclusive. The same author also cut the vomeronasal nerve unilaterally and the photomicrographs of the postoperative epithelial tissue showed no apparent regeneration.

Schultz (111) reported on the changes in monkey olfactory mucosa after experimental destruction with zinc sulphate, but described abnormalities in the form, orientation, distribution, and population density of the presumptive "restored" cell bodies. This was a preliminary report but we have found no subsequent records.

Negative evidence has been reported from several sources for several classes. In three species of fish, Quastler and Weingarten (112) found no evidence of olfactory cell regeneration after cauterization of the olfactory area. In three species of a microsmatic lizard, Armstrong and co-workers (113) found that following extirpation of the olfactory and accessory bulbs the epithelial sensory cells degenerated and that there was some fragmentation and loss of fibers in the olfactory tract. Repair was not discussed.

In the rat, Takata (114) found that after transection of the olfactory fila

there was no regeneration of olfactory cells, no mitosis, and no regeneration of the fila, that Bowman's glands were initially shrunken and recovered slowly, and that in the event of severe damage olfactory epithelium was replaced by ciliated epithelium.

In the rabbit, Le Gros Clark (115) observed that destruction of olfactory epithelium was followed by complete degeneration of the fiber plexus on the surface of the bulb. The fascinating phenomenon of the survival of about half of the sensory cells even after extirpation of the olfactory bulb is also discussed, and it is pointed out that a good deal of interaction between immediately adjacent axons in the Schwann cell sheath may occur during the passage of impulses. This paper is one of a related series on the structure and organization of receptors which cannot be done justice by précis. The survival phenomenon underlies Kolmer's observation (2) that it is difficult to distinguish whether restoration of olfactory capacity depends on the regeneration of mucosal elements or on functional substitution of an intact portion for a destroyed portion.

The experiments of Stuart-Harris and Francis (15, II) on the differential effects of zinc ionization on the nasal tissues of normal and influenza-convalescent ferrets clearly describe the destruction of both respiratory and olfactory epithelia, but unfortunately for our context they trace the regeneration of the respiratory portion only and the fate of the olfactory is not recorded.

Perrin and Oliphant (116), reporting further on the histology of the same virus infection in ferrets, described necrosis and desquamation of some of the superficial epithelial cells in the olfactory regions of infected animals; beginning on the fourth day, olfactory involvement varied from animal to animal, the affected areas were small to moderate in size, and at least half and at times three-fourths of the mucosa remained intact. They did not specify whether the *sensory* cells were desquamated or damaged, noting that as the "superficial cells" sloughed off "deeper cells" were swollen and without polarity. Leucocyte infiltration was rarely as dense in the olfactory as in the respiratory lamina propria. By the nineteenth day the olfactory epithelium looked essentially normal; again no reference was made to the sensory cells.

Hyperplasia of the olfactory glands was illustrated clearly in experimental infection of the chick with Newcastle disease virus (16). It is also evident in the coccobacilliform infection of adult chickens (see Fig. 11). Maxillary sinus epithelium is known to regenerate following both surgical (117, 118) and experimental (119) resection; the role of the special cells described by Bast (84) has evidently not been studied during regenerative growth.

Experimental efforts to "coat" mucosal surfaces in order to render them resistant to viral infections had a temporary popularity, but neither oil, antiseptics, alum, nor tannic and picric acids proved feasible except as quite transitory and variable inhibitors; the first two indeed have been designated as more objectionable than beneficial (96, 120). Attempts to absorb virus particles

onto chemicals instilled into the nasal chamber have been equally doubtful (121). Specific effects of these chemicals on olfactory mucosa were not mentioned.

DISCUSSION

A comparison of results of experimental upper respiratory infections of different laboratory animals may be difficult. The normal chemical properties of the groups of paranasal and paraorbital glands which pour secretions into the nasal chambers have not been systematically analyzed, nor have the role of these glands under infectious or physiological stress.

The olfactory sensory epithelium is directly served by the olfactory glands (Bowman's) in all land vertebrates, and anatomically could be subserved by the maxillary gland secretions of several species of birds and mammals. The paraorbital (lacrimal and Harderian) gland apparatus discharges directly onto the olfactory mucosa in lizards and snakes; whether these several fluids simply serve to keep the receptor fibrils washed or whether they mechanically or antibiotically protect the area from trespassing particles, or all of these things, has not been established. If damaged olfactory cells cannot regenerate in adult animals, protection of this area in macrosmatic species may be essential for survival in the wild.

The anterior, respiratory, section of the nasal chamber of land vertebrates receives fluids directly from the epithelial acinous and goblet cells, from the lateral nasal gland, from the lacrimal duct, and in forms with highly erectile vomeronasal tissues, from the vomeronasal organ. Again the normal and induced biochemical properties of the secretions have not been precisely defined; their reactions may well be mutually stimulated or reinforced.

The olfactory organ, in adjusting to joint use for respiration and thence to enormous environmental differences, has undergone extremes of variation in location, proportion, architectural support, and impact engineering but has retained its primitive structure and an open contact with its medium. The safeguards which have been acquired as insurance for food-finding and reproduction, and insurance against the depredations of enemies and contaminants, should perhaps be fully taken into account in interpreting host tissue responses to upper tract disease. Kelemen (126) emphasized the prevalence of spontaneous pathological changes in the nasal cavities of laboratory and farm stock species. In a series of studies recently summarized (127) he pointed out that evaluation of results of functional experiments must allow for this frequent (60% in rats) condition in "normal" stock.

SUMMARY

Part I

The anatomy of the nasal chamber of the laboratory chicken, rat, and ferret has been described. The significance of the area in infectious diseases of the

respiratory tract has been considered. The relationships of the sources of fluids which discharge into the anterior chamber and of the structures principally concerned with olfaction and/or respiration are described and illustrated. Comparisons with examples of adaptive variations in a few related species of animals are illustrated.

Part II

Common factors in the phylogeny and development of the vertebrate organ of olfaction, and the adaptive variations of the organ to joint use as a respiratory organ are considered. With their possible significance in nasal chamber pathology as a focus, the complex of intra- and paranasal structures of fish, amphibia, reptiles, birds, and mammals are reviewed structurally; in each case in which a known function could be demonstrated or indicated, such evidence is presented.

The olfactory sense in relation to the pathology of the nasal chamber is reviewed and discussed. The repair of nasal mucous membranes, and efforts to protect them against infection, are reviewed in the same context. The ensemble of functions of the paranasal tissues is considered in relation to the use of laboratory animals in experimental infections of the upper tract.

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ABSTRACTS OF PAPERS PRESENTED BEFORE THE MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

December 8, 1958

CONTROLLED STUDIES IN THE TREATMENT OF CANCER

LOUIS LASAGNA AND ALBERT H. OWENS, JR.

At the present stage in the history of cancer chemotherapy, controlled clinical trials seem indicated for the following reasons: 1) there is no excellent drug yet available; 2) there are semantic problems relating to the definition of "remission", "success", "failure", etc.; 3) greatly varying claims as to efficacy of drugs continue to appear; 4) many "oncolytic" drugs are extremely toxic, so that one is frequently faced with the need for weighing minor therapeutic gains against major therapeutic mischief; and 5) the great need for evaluating the validity of laboratory "screens" demands reliable and quantitative data in humans as well as in animals.

The Eastern Cooperative Solid Tumor Group, composed of representatives from six hospitals along the eastern seaboard, has compared nitrogen mustard (HN_2) and triethylenethiophosphoramide in 258 patients suffering from carcinoma of the lung, carcinoma of the breast, melanoma, or Hodgkin's disease. The experiment was conducted along the principles of the classical controlled trial.

The randomization procedures utilized appeared to achieve comparable therapeutic challenges in the various disease subgroups. Diminution in size of single tumors, in the absence of diminution of total tumor mass, was not considered evidence of anti-tumor effect. The drugs achieved significant reduction in total tumor mass in 16 per cent of the 258 patients treated, and "general benefit" other than reduction in tumor mass in an additional 4 per cent. The only significant differences between treatments occurred in the patients with Hodgkin's disease, where diminution in size of lesions and duration of response was greater in the HN_2-treated group. No differences between drugs in effect on median survival time were seen in any category. Serious toxicity was observed with both drugs to about an equal degree, and occurred almost exclusively in patients subsequently classified as "failures".

The implications and lessons of the study were discussed.

SOME EPIDEMIOLOGICAL ASPECTS OF CERVICAL CANCER

ABRAHAM M. LILIENFELD

One of the most generally accepted facts concerning cancer of the cervix is its low frequency among Jewish women. Studies of hospital patients in the United States and a comparison of frequency of cervical cancer in Israel and the United States have been consistent in indicating such a difference. This has led to the development of the hypothesis that circumcision of the sexual partner might have a preventive effect on the occurrence of cervical cancer. To directly test this hypothesis, Wynder obtained information by interview concerning circumcision status of husbands from a group of cervical cancer patients and controls in several hospitals. In addition, he studied the possible influence of such variables as age of first marriage and/or coitus and number of pregnancies. With respect to circumcision, Wynder found that a larger proportion of cervical cancer patients stated that their husbands were uncircumcised than did the control group. Expressing this relationship in terms of relative risks he indicated that women with a circumcised husband had a 60 per cent less

risk of developing cervical cancer than did those with an uncircumcised husband. Regarding age of first coitus, he noted that a larger proportion of cervical cancer patients gave a history of having had first coitus before the age of 16 than did the controls. Wynder also confirmed the frequently observed increase of cervical cancer with increasing number of pregnancies. Since number of pregnancies is independently related to age of first marriage he adjusted for each of these factors and determined that the important variable was age of first marriage and not the number of pregnancies. From these findings, Wynder developed the concept that a factor associated with lack of circumcision, possibly Smegma, was of etiological importance and that variations in frequency of the disease in different population groups depended upon the presence of this factor and also upon the length of time a woman was exposed to this factor; this would explain the association of cervical cancer with earlier age of first coitus. Such a concept was consistent with the results of other studies which have indicated an association of cervical cancer with syphilis and prostitution and the suggested rare occurrence of cervical cancer among nuns.

Since Wynder's study was carried out on a hospital patient population which may be select in many ways, we consider it desirable to test the circumcision hypothesis in a study in which all cervical cancer cases in a community were included together with a population sample as a control group; such a study was initiated in 1956 in Buffalo. Early in the study, the interviewers had the "clinical impression" that wives were uncertain regarding the circumcision status of their husbands even though they provided a "yes" or "no" answer to this question. We considered it necessary to determine the validity of this type of information by a separate study. The best method of validation would be a comparison of the wife's answer with results of examination of her husband. In view of practical difficulties in such a study, we thought that a first approach could be made by a comparison of results of examination with a person's statement concerning his own circumcision status. If a fair amount of disagreement were noted, one would expect somewhat more disagreement when wives' statements were compared with examination results. Such a study was carried out at the Roswell Park Memorial Institute during January–March, 1958.

Comparison of statements of 213 male patients with examination findings indicated disagreement between examination and statement in 34.4 per cent of the patients. Persons whose statements agreed and disagreed with examination were similar in birthplace, languages read and spoken, socioeconomic category of occupation, and age. Analysis of the proportion of disagreeing patients examined by each of six physicians indicated general similarity. Characteristics of patients who stated they were not circumcised, when the physician determined they were circumcised, were not significantly different from those who reported circumcision when the examiner recorded the reverse. However, the first-mentioned group was consistently different to a slight extent from the other in characteristics suggesting less familiarity with the English language. Also, there was a smaller proportion of professional workers in this group, and they were somewhat older.

It is difficult to evaluate the results of this study to Wynder's findings. Assuming that the same degree of disagreement in Buffalo was also present in Wynder's group and correcting Wynder's results to take into account this disagreement, it was observed that the frequency of circumcised husbands among the cervical cancer group is similar to that in the control group.

The present results should not be interpretated as invalidating the circumcision hypothesis. The most that can be said is that the hypothesis has not been adequately tested. There is a distinct need for a study in which husbands of cervical cancer patients are examined and compared with husbands of a control group.

TOBACCO AND MORTALITY FROM LUNG CANCER AND OTHER CAUSES

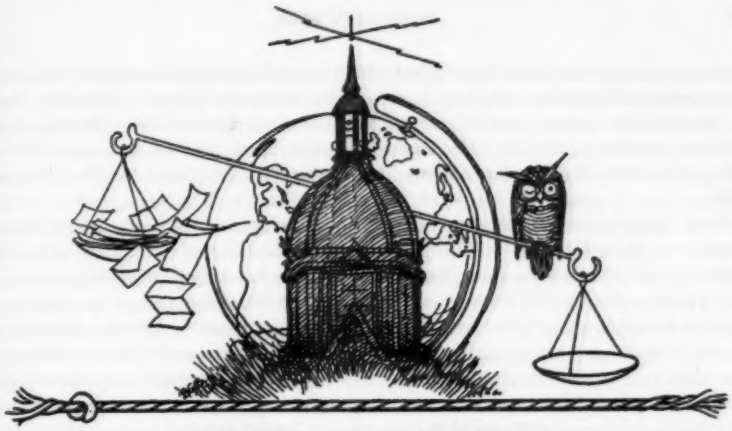
JEROME CORNFIELD

The epidemiological evidence implicating tobacco, largely in the form of cigarettes, in the development of lung cancer is of several types. (1) In over 20 independently conducted retrospective studies the proportion of cigarette smokers among those with lung cancer has been well above the proportion found in the non-lung cancer groups. (2) In three independently conducted prospective studies, two in this country, and one abroad, the mortality from lung cancer among those originally classified as cigarette smokers has been tenfold that of the non-smokers.

Mortality from several other causes, particularly cardiovascular disease, is also found to be associated with cigarette consumption. The relative excess in mortality from cardiovascular disease among cigarette smokers is less than that for lung cancer, but because of the importance of cardiovascular disease as a cause of death, the absolute excess is greater.

Condensed tobacco tars have induced skin tumors in several strains of mice and in rabbits, and lesions in the respiratory tract of dogs. Tobacco smoke has produced respiratory lesions among chickens and mice.

The evidence implicating cigarettes as a major factor in the etiology of epidermoid carcinoma of the lung can no longer be lightly dismissed.



BOOK REVIEWS

The Johns Hopkins Hospital and The Johns Hopkins University School of Medicine. A Chronicle. Vol. II, 1893–1905. By ALAN M. CHESNEY. 499 pp., $6.00. The Johns Hopkins Press, Baltimore, Md.

The publication of the second volume of the history of the Johns Hopkins Medical School and Hospital is an event of the first importance in the life of these institutions; the work itself makes history. For just as a fine piece of research becomes doubly valuable when published for all to read and analyze, so the elements that went into the making of a great medical school gain significance by being recorded in the perspective of time. Writing from a base of sound historical scholarship and profound knowledge and love of his subject, Alan Chesney has produced a work which is informative and revealing without being tedious; light in its touch without being superficial.

The first volume dealt principally with the initial plans for the Hospital and the Medical School, and with the early days of the operation of the Hospital. This second volume covers the period from the opening of the Medical School until Dr. Osler left for Oxford. Of the faculty and students of those years, a few are happily still with us, and most of them indeed were familiar figures to all but the youngest generation of Hopkins men.

The book is truly a chronicle of the persons and events of those years; but the homely approach is disarming, for out of the official and personal correspondence of Drs. Welch, Osler, Kelly, Mall and many others, the minutes of the Advisory Board of the Medical Faculty, and material from the School Catalogues of the day, there emerges a clear picture of men and women who instinctively felt that they were pioneers, who sensed that what they were doing was of enormous importance. And so it was, for in this period the great idea outlined by Gilman and Billings was put to the test, and one can see how the practical decisions, many no doubt significantly influenced by the junior members of the faculty, gave form and direction to the evolving curriculum and philosophy of medical education.

Much that is recorded here will be of interest largely to those who have had some connection with Johns Hopkins and Baltimore, or with one or another of the principal actors. Beyond this, however, there is a wealth of material bearing on the problems of medical education, and many of those problems are still with us today.

Here we see Mall, often through the eyes of some of his early students, instituting not a "course" in anatomy but *instruction* in anatomy in a pattern which has profoundly influ-

enced every generation of Hopkins students down to and including the present. Among other features established in the curriculum in those early days and still highly valued at Hopkins were concentration on only one or two subjects at a time, de-emphasis of formal examinations, the provision of elective or free time, and encouragement of student research. It was at this period, too, that the first endowed lectureship was established by Dr. Christian A. Herter.

Clinical clerkships on the wards of the Hospital, an educational feature now taken for granted, was distinctly novel in America at that time and indeed constitutes one of Johns Hopkins' great contributions to medical education. It is interesting to find accounts of prolonged consideration by the Faculty and Trustees in 1898 of the manner in which professional fees from private patients seen by the full-time staff should be handled. Even in those early days there was an appreciable number of patients who came to the institution itself rather than to a particular physician. Fees collected by the Hospital for professional fees rose from $850.00 in 1898 to over $11,000.00 the following year.

The Medical School and Hospital had its amusing incidents and its teapot tempests, too. As an example of the former, Dr. Welch, having been Dean of the Medical Faculty for two years, at a meeting of the Advisory Board from which Dr. Osler was absent, resigned and accomplished the election of Osler as Dean. The latter refused to serve, and Welch's coup having failed, he continued as Dean for another three years!

Chesney tells, too, of the minor crisis over what is generally known as the Anatomy Building, the gift of a great benefactor of the Medical School, Miss Mary Garrett. President Gilman's intention to place an appropriate plaque inside the building collided squarely with the wishes of this indomitable lady, who specified that the building should be labelled by a prominently displayed sign, Women's Fund Memorial Building. She not only had her way but saw to it subsequently that the building was so designated in all official communications.

Through the pages of this easy-going and delightful book move not only the senior faculty but the many younger men and women who later made medical history in Baltimore or elsewhere in America. The book is profusely illustrated with well selected photographs and sketches, an appendix contains class and faculty rosters, and there is a serviceable index.

To this reviewer the second volume of Alan Chesney's History is even more interesting than the first and equally valuable for its basic historical material on medical education in America. In this period the high purpose for which the Johns Hopkins School of Medicine and Hospital were founded was at last being approached. In apposition to a handful of distinguished professors were now placed a growing group of alert students and junior faculty from all over the United States and Canada. One can sense the excitement of this new community in which teaching and research and patient care were inseparably blended. It is easy to believe that faculty and students alike were calmly conscious of the destiny of the development of which they were a part. It is a happy thought that most of them lived to see this destiny fulfilled.

THOMAS B. TURNER

Deficiency Disease. By RICHARD H. FOLLIS, JR. 577 pp., $14.75. Charles C Thomas, Springfield, Ill.

This book is an excellent account of our accumulated knowledge of how mammals are affected by lack or insufficiency of each of the essential nutrients. The subtitle: Functional and structural changes in mammalia which result from exogenous or endogenous lack of one or more essential nutrients, accurately describes its contents. It is the outgrowth of a monograph, The Pathology of Deficiency Disease, which was published in 1948, which dealt almost entirely with experimentally produced nutritional syndromes in laboratory animals.

The present work describes and interprets the pathological states resulting from single nutrient deficiencies, usually experimentally produced, and also those resulting from lack of multiple nutrients. This objective broadens the scope of the discussions to include all conditioning factors which might produce general or local reduction or deficiencies of essential nutrients. Inanition, excess of food, and metabolic disorders of varied natures arising from deficiency or imbalance of nutrients are discussed from the standpoint of functional and anatomical changes in cells, tissues and organs. All illustrations are well chosen and are of good quality.

The scope of the work includes the significance of sixteen inorganic elements, of individual amino acids, and of protein deficiency in general, the essential fatty acids, the carbohydrates, seventeen vitamins. These are discussed in Parts I-VI. Part VII treats of the naturally occurring deficiency diseases. It covers twenty-two nutritional disorders, their causes and prevention. In Part VIII the pathologic physiology and anatomy of specific tissues in deficiency diseases are described. Lastly, there is a discussion of deficiency disease as a research method in biology and medicine. The reader will be impressed by evidences of scholarship on every page. It seems to this reviewer almost incredible that the author was able to gain mastery of details derived from the fifteen hundred and thirty-five scientific papers which are cited in the bibliography, and to write such an interesting and informative an account of the researches of thirty-nine hundred and two investigators.

The author has achieved remarkable success in telling with clarity what every biochemist, physiologist, pathologist and clinician desires to know about the present status of our knowledge of how each nutrient participates in metabolic processes, and the nature of the basic problems involved in the most important problem, the health of man and domestic animals throughout the world.

E. V. McCollum

Obstetric and Gynecologic Milestones. Essays in Eponymy. By Harold Speert. 700 pp., $15.00. The Macmillan Co., New York, N. Y.

This is a highly unusual attempt to portray certain highlights of obstetrical and gynecological history by a series of short treatises on various men whose names have become more or less an integral part of our respective specialties. The 700 pages are divided into 79 chapters, although some of these are devoted to more than one individual. Liberal illustrations are included, and these are amazing in that they comprise a considerable number of fine woodcuts, plates, prints, and photographs. Just how these could be accumulated by any one author is in itself worthy of a book.

The 79 chapters can be regarded as a compendium of "separate essays independent of one another". These eponyms are in a sense a record of ob-gyn. development as independent specialties and include the years from Gabrielle Fallopio (1523–62) up to the recent era of Joe Meigs or Stein and Leventhal. The obstetrician will find a resume of the particular work and a short summary of such men as Scanzoni, Mauriceau, Crede, Ritgen, Porro or Duhrssen. The gynecologist will be interested in similar treatment of such names as Latzko, LeFort, Watkins and Wertheim. To simply illustrate a typical chapter, this involving the paramesonephric duct, the author presents a short life history of Johannes Müller with a detailed history and observation as well as a brief resume of other pertinent work.

The author has seen fit to divide into nine rather uneven parts his 79 chapters as follows: Part I (25 chapters) Anatomic Parts, Landmarks and Measurements, II (10) Clinical Signs, Tests and Rules, III (6) Phenomena of Pregnancy and Labor, IV (2) Bacteria, V (2) Ovarian Tumors, VI (4) Syndromes, VII (3) Positions, VIII (10) Instruments and IX (17) Operations and Therapeutic Procedures. It is doubtful whether any purpose is accomplished by

such an unbalanced and incomplete division into separate parts, and individual preference would allow each chapter to exist as the separate entity it purports to be.

There are those of us who remember Harold Speert as a diligent and extremely intense Hopkins interne in past years. This is exactly the type of book I would expect him to accomplish, although there is a curious omission of such Hopkins and world famous eponyms such as Kelly (cystoscope), Hunner (ulcer or interstitial cystitis), Emil Novak (suction curette) or Richardson (composite operation) to mention only a few. On the other hand, Cullen's sign is noted, and it should not be construed as disrespectful to our late professor if this reviewer states that this entity is not pathognomonic of ectopic pregnancy as well as almost as rare as an Oriole sweep of a Yankee double header. Obviously, however, Speert had to draw some limit as to what he could include in his material.

As to the appeal of this book, it is difficult to say, although there can be no question that it is well done and represents a huge amount of time and labor. Certain chapters were tremendously interesting to me; I suspect that a few (may I say discriminating) readers will be completely intrigued by occasional parts of this text. In toto, however, and to most readers, it will arouse no particular interest. It is this reviewer's suspicion that the author, in preparing the text, was well aware of this, but could not resist the challenge of something so unique and exotic in the field of medical literature.

EDMUND R. NOVAK

Epilepsy. By MANFRED SAKEL. With a Preface by Otto Poetzel. 204 pp., $5.00. Philosophical Library, New York, N. Y.

One, accustomed to reading current physiological discussions of epilepsy, upon turning the pages of this volume might well exclaim with Alice in Wonderland "Curiouser and Curiouser". For here are the characters of physiology in psychiatric garb. Thus we read of "relaxation oscillation" "tonus catch" "epileptic time—lapse—motion" "wander-waves" and "catharsis of the pre-epileptic excitation mass" in the preface to the manuscript of Manfred Sakel, originator of insulin "shock" therapy. This work was written by Sakel just before his death, and never completed. The original material was not altered, although "it has been annotated and brought up to date under the guidance of several neurologists and psychiatrists".

In the first half of Sakel's manuscript, a concise and orthodox clinical description is given of the epilepsies and their treatment.

The second half of the book, which was not completed, is devoted to the discussion of Sakel's thesis that "the idiopathic convulsion is caused by the sudden appearance of a steep difference between the tonus of the vagus and the sympathicus," which he thought might be prevented by permanent overstimulation of the sympathicus. This he hoped to achieve by causing a permanent overactive functioning of the thyroid gland by grafting "Basedow thyroid tissue" into the epileptic.

A detailed account is given by three patients, two of whom had such grafts implanted in the thyroid gland. One is said to have been rendered free of attacks for a year or more and to have been improved mentally. Only a rough draft of the discussion of these cases seems to have been completed by Sakel.

The humanist will find this little volume a fascinating study of the thinking of a psychiatrist who tried to analyze what he observed.

A. E. WALKER

Emergency War Surgery, NATO Handbook. 411 pp., $2.25. United States Government Printing Office, Washington, D. C.

This book is a short, simple, well written manual prepared for use by the Medical Services

of the NATO Nations. The handbook is arranged with a large list of contents, numerous, rather short chapters, and a good index. Many aspects of traumatic surgery including all the surgical subspecialities are included in the text. Obviously, therefore, consideration of each aspect is brief and didactic. This is necessary because of the scope and intent of the text. In addition to the general topics in this manual, there is considerable mention of thermonuclear warfare. A section in each chapter is devoted to the management of mass casualties of the particular type under discussion.

This book is particularly recommended for those who are concerned with military medicine and especially those working in forward combat areas.

This handbook would also be of value for medical students and house officers in emergency rooms.

WILLIAM F. RIENHOFF, III

Schizophrenia. By MANFRED SAKEL. 335 pp., $5.00. Philosophical Library, New York, N. Y.

Sakel introduced the insulin shock treatment of schizophrenia in 1927. This book is an elaborate and somewhat repetitious summary of his experience with the treatment, published a year after his death. The volume is divided into two parts of equal length: 1) Etiology, Symptomology and Psychopathology; and 2) The Sakel Insulin Shock Treatment. There is a meager one page index of authors referred to in the text.

Insulin shock treatment for schizophrenia was one of those empirical discoveries in clinical medicine which was met with spirited opposition. Sakel, using large doses of insulin to deal with the withdrawal symptoms of addicts, thought he might also affect the symptoms of schizophrenia. He found that he could alter the course of that disease, often for the better. The importance of his discovery lay in the idea that schizophrenia was curable, even if insulin shock was not exactly the cure Sakel felt it was.

An astute observer and clinician, he also developed a set of hypotheses about the nature of endogenous psychoses. While at times these ideas seem somewhat fanciful, again their larger value would seem now to be the stimulation these hypotheses provided for a rethinking of our concept of such illnesses.

JEROME HARTZ

Neurological Basis of Behavior. Ciba Foundation Symposium. Edited by G. E. W. Wolstenholme and Cecilia M. O'Connor. 400 pp., $9.00. Little, Brown & Co., Boston, Mass.

Prior to the meeting in Brussels, in 1957, of the various international congresses in the neurological sciences, the Ciba Foundation invited a group of 30 scientists to participate in a symposium on the neurological basis of behavior. One of the purposes of the meeting was to commemorate the centenary of the birth of Sir Charles Sherrington. Anatomists, physiologists, psychologists, neurologists, and neurosurgeons were present.

In this volume several authors review the behavioral effects of transecting the brainstem and ablation of cortical and subcortical structures. Bard describes the rather complex motor activity that can be elicited from an animal with a transection of the mesencephalon. Klüver discusses recent thoughts on bilateral temporal lobe ablation in monkeys (Klüver-Bucy syndrome) and Green presents evidence that two of the behavioral components of this syndrome, hypersexuality and hyperphagia, can be produced in isolation by discrete lesions in the medial temporal lobe.

Of special interest in regard to motivational behavior are the experiments of Olds in which he maps areas of the brain which a rat will self-stimulate with an implanted electrode, by pushing a pedal (positive motivational effect) and those areas with electrode implants which

the animal avoids stimulating (negative motivational effect). The positive motivational field centers on the hypothalamus and includes most of the rhinencephalic cortex.

Gastaut discusses Pavlov's hypothesis that conditioned reflexes are established by the "closure" of a circuit between conditioned and unconditioned analyzers in the cortex and then presents his own detailed hypothesis that "closure" occurs within the midbrain and thalamic reticular systems. However, projections from subcortex to cortex are necessary for elaboration of the characteristic motor responses. Jasper tends to confirm this hypothesis with micro-electrode studies of units in the motor cortex before and after conditioning with a light flash. It is their impression "that it is necessary to look elsewhere than motor cortex for convergence of impulses critical to the establishment of neural pathways of the conditioned response."

In recent years there have been several contributions to this field which receive only casual mention. Sherwood discusses the effects of injecting a variety of drugs in the lateral ventricles of animals and man, but some of the most significant advances in neuropharmacology, the effect of psychotomimetic and tranquillizing drugs in animal preparations and man, are not considered. This book continues the trend toward de-emphasizing the importance of the cortex as "the final common pathway" of behavioral expression, although it is frequently noted that the cortex is necessary for a co-ordinated response. There is good reason to believe that human behavior, behavior based on intellect, is possible because of the size of man's cortex, and a defense of this hypothesis, many of which have appeared in recent years, might have added to the scope of the symposium.

The papers in this volume are unusually succinct and the discussions are particularly enlightening. Its great value is in the presentation of cogent data and hypotheses by the world's leading authorities in this rapidly changing field.

THOMAS W. LANGFITT

Eye Surgery, 3rd edition. By H. B. STALLARD. 899 pp., $18.00. The Williams and Wilkins Co., Baltimore, Md.

The third edition of Eye Surgery by H. B. Stallard will be welcomed by those familiar with his two earlier editions. The book has been extensively revised and much new material added. The author's original purpose of describing fully the details of eye operations accepted in current practice and discarding obsolete operations has been admirably adhered to. The text is clear and explicit. The illustrations are excellent. This is one of the finest textbooks on eye surgery available.

HOWARD NAQUIN

Physical Examination of the Surgical Patient, 2nd edition. By J. ENGLEBERT DUNPHY AND THOMAS W. BOTSFORD. 375 pp., $8.00. W. B. Saunders Co., Philadelphia, Pa.

In this second edition of a noteworthy addition to the library of surgical writings a quotation from Dr. William Thayer precedes the foreword: "Learn to see, hear, feel, and smell and know that by practice alone you can become an expert". With this attitude the authors lead the student or practitioner gently but persistently into the avenues of careful, cogent observation, laying a groundwork of anatomy and pathology to precede the discussion of each region of the body.

This book maintains the pleasant tone and tutorial air of Cope or Homans. Indeed, many of the illustrations are from Dr. Homans textbook. This literary style, and the copious illustrations make the text a pleasant exercise in reading alone. It is, however, the depth of experience of the authors, and their sophistication as teachers that makes it succeed in conveying such a wealth of material successfully.

As textbooks go, this one is short. It is divided into twenty chapters. The first chapter deals with history taking and points out the essentials of a "surgical" history. Chapter 14 is a new chapter on "Special Examination for the Detection of Cancer". The final six chapters deal with injuries. While each chapter is introduced with appropriate anatomy and pathology, the temptation to discuss therapy is avoided, and special examinations are disdained lest they distract from what use of the senses can reveal. General remarks concerning examination of the region are followed by lucid, concise descriptions of physical findings in the common surgical afflictions involving the area. A paucity of verbage carries the reader forward with a definite momentum. This critical editing by the authors has enabled them to compress an enormous amount of material into a small space, a virtue too infrequently encountered in medical texts.

While the rather didactic presentation may prove irritating to the accomplished surgeon of experience, the student of physical diagnosis, whatever his echelon, will find this an engrossing and valuable vade mecum which transmits the enthusiasm of its authors most effectively.

J. C. HANDELSMAN

Physiology of Prematurity. Transactions of the Second Conference, March 1957. Edited by JONATHAN T. LANMAN. 160 pp., $3.75. The Josiah Macy, Jr. Foundation, New York, N. Y.

The Transactions of these relatively unstructured meetings are virtually verbatim accounts of the interchange of ideas, opinions, speculations, and information among about 25 participants who bring to bear on the subject under discussion a multidisciplined background.

The Second Conference on Physiology of Prematurity was given over to a discussion of "Aerobic and Anaerobic Metabolism in the Fetus and Newborn" led by Claude Villee from the Department of Biological Chemistry of Harvard Medical School and to "Breathing and its Control in Premature Infants" led by Petter J. E. Karlberg of the Pediatric Clinic of Karolinska Institutet. It seemed to this reviewer that there was less of the spontaneous informal 'give and take', or put another way, a reduced exchange among the participants than we have come to expect in a Macy conference. It seemed also that the discussion was somewhat less incisive than customary. Several examples of this might be cited: the one which seemed most glaring had to do with the exchange of lactic acid from the human fetus in utero and the maternal circulation—the presentation on pages 67 through 70 appears to indicate production of lactate by the fetus, extraction by the placenta, and subsequent passage of at least a part of the lactate to the maternal circulation, then much to our surprise—on page 71 we find the statement which goes unchallenged that the infant cannot transfer lactic acid across the placenta into the mother. This reviewer would also like to question the propriety of the word "control" in the title of the second portion. Perhaps a better title might have been "The Mechanics of Breathing in the Newborn."

As reference works for students (in the broadest sense) the Transactions are unique and invaluable sources, not only of information but also of stimulation. The Transactions never fail to provoke in a thoughtful reader a multitude of questions to be answered by further research.

GILBERT J. VOSBURGH

Analyzing Psychotherapy. By SOLOMON KATZENELBOGEN. 126 pp., $3.00. Philosophical Library, New York, N. Y.

Many patients in out-patient psychiatric treatment have the impression that they are in "psychoanalysis", when, more exactly, they are having psychotherapeutic conferences. Dr.

Katzenelbogen's book is an attempt to clarify, for interested people who are not professional psychiatrists, some of the distinctions between the two. He further attempts to put into the book something of what he does in psychotherapeutic sessions and why. In general, eclectic psychotherapeutically oriented psychiatrists do do approximately what Dr. Katzenelbogen does, for approximately the same reasons, and with the same sort of patients (although most would attempt to use more currently "okay" or "dynamic" words and concepts). It is certainly time that information of this sort were made generally available, and somewhat surprising that there are not many such efforts on the market. It would seem that few psychiatrists are anxious to advertize that they are not what Dr. Katzenelbogen calls "patented psychoanalysts", so that only someone of his stature would attempt a book of this sort.

Most of us would naturally quibble about a few of Dr. Katzenelbogen's concepts and tactics, but, quite apart from these minor matters, the book might have been made even more useful. It is written in a lucid, intelligible, conversational manner, but the literary style leaves something to be desired. The phraseology is sometimes clumsy, and the last half of the book is so loosely organized that it appears to have been dictated, sent off, and published without revision or proof-reading. Sophisticated patients might be relatively unimpressed. Perhaps a professional writer could have helped. Dr. Katzenelbogen's publishers also took the liberty on the book jacket of promoting him from former "Associate Professor" here to former "Professor of Psychiatry at Johns Hopkins Medical School".

In spite of these latter comments, this is a currently useful book, recommended for selected patients considering psychiatric treatment or skeptically in it, and also for medical students treating out-patients in psychiatric clinics.

FREDERICK J. ZIEGLER

Electrocardiography. By MICHAEL BERNREITER. 134 pp., $5.00. J. B. Lippincott Co Philadelphia, Pa.

This book is a very concise but adequate book on electrocardiography. The emphasis is on clinical application with excellent figures of electrocardiograms in the various conditions described. A minimum of space is devoted to theory and physiology.

The book is keyed to the medical student or general practitioners level. I believe it is an easy book to begin one's training in this field and is written with great clarity. In a minimum of space the highlights of electrocardiography are covered. The book makes no pretenses at being a complete analysis of the subject. I think this would be a very desirable and valuable book to have available to medical students and housestaff members as an introduction to electrocardiography.

RICHARD D. FRITZ

The Psychology of Medical Practice. By MARC H. HOLLENDER. 276 pp., $6.50. W. B Saunders Co., Philadelphia, Pa.

The purpose of this book, nicely outlined in the introduction, is "to turn a psychological spotlight on the patient, the physician and what might be called the medical situation" in order to "evolve general principles and to illustrate an approach". Its aim is eminently practical and has been well achieved. The psychologic presentation is good despite an occasional tendency to oversimplify interpretations of motivation. The author is assisted by three contributors who are specialists in clinical medicine, obstetrics and gynecology and pediatrics.

The dynamic viewpoint is basic and the emphasis placed on the interpersonal relations between the patient and the physician is sound. The importance of seeking to understand the meaning of things done by the patient is well illustrated with practical examples. In

some cases, a running account of conversation between patient and doctor and also between doctor and the close relative of the patient helps to illuminate the approach to better therapy. There are chapters applicable to the medical, surgical, obstetrical and pediatric patient. The last two chapters deal with the psychological considerations in the use of medications and with the dispensing of the non-medicinal prescription.

A number of philosophies of life are expressed in the discussion of patient problems; these are especially enlightening in the chapter on "The Patient with Carcinoma". As regards the aspect of religious belief and the problem of the psychologic stress that conflicts and misconceptions might provoke, there is limitation of comment, perhaps discreet. From the psychiatric standpoint, questions involving the obstetrical patient are particularly well treated and might well be consulted by readers of the other sections if only to gain more of a feeling for the various trends of conscious and unconscious mental processes affecting the patient.

This is an instructive book and should be valuable at least in some of its parts for physicians at all levels of medical practice.

EDWARD L. SUAREZ-MURIAS

Ciba Foundation Symposium on The Cerebrospinal Fluid. Production, Circulation and Absorption. Edited by G. E. W. WOLSTENHOLME AND CECILIA M. O'CONNOR. 335 pp., $9.00. Little Brown & Co., Boston, Mass.

The symposium on the cerebrospinal fluid was held at the Ciba Foundation in London, England May 27–29, 1957. The speakers were mainly from England, only six being from the continent and two from North America. The discussion, therefore represents largely the current English concepts of the fluid surrounding the brain and spinal cord. These opinions have a slightly different orientation than those currently held in American neurological circles and hence this volume should be of interest to all students of the nervous system in this country.

A. E. WALKER

An Atlas of Airborne Pollen Grains. By H. A. HYDE AND K. F. ADAMS. 112 pp., $10.00. St Martin's Press, New York, N. Y.

This book provides an excellent atlas of pollen grains encountered in pollen surveys in England. The illustrations are clear. The indexing is complete.

Most pollen grains encountered in the New England and Middle Atlantic states in the United States are illustrated. However, important pollens found in Southern, Midwestern, and Western states are not described because they are not encountered in surveys conducted in England.

This book is recommended to anyone responsible for identifying pollen grains.

T. E. VAN METRE, JR.

BOOKS RECEIVED FOR REVIEW

Biological and Biochemical Bases of Behavior. Edited by HARRY F. HARLOW AND CLINTON N. WOOLSEY. 476 pp., $8.00. The University of Wisconsin Press, Madison, Wis.

Cardiac Arrest and Resuscitation. By HUGH E. STEPHENSON. 378 pp., $12.00. The C. V. Mosby Co., St. Louis, Mo.

The Chemical Prevention of Cardiac Necroses. By HANS SELYE. 235 pp., $7.50. The Ronald Press Co., New York, N. Y.

Clinical Epidemiology. By JOHN R. PAUL. 291 pp., $5.00. The University of Chicago Press, Chicago, Ill.

Cold Injury. Transactions of the Fifth Conference March, 1957. Edited by M. IRENE FERRER. 341 pp., $5.95. The Josiah Macy, Jr. Foundation, New York, N. Y.

Cyclopropane Anesthesia, 2nd edition. By BENJAMIN HOWARD ROBBINS. 293 pp., $9.00. The Williams & Wilkins Co., Baltimore, Md.

Nurse-Patient Relationships in Psychiatry, 2nd edition. By HELENA WILLIS RENDER AND M. OLGA WEISS. 319 pp., $5.95. McGraw-Hill Book Co., New York, N. Y.

Physiology of Spinal Anesthesia. By NICHOLAS M. GREENE. 195 pp., $6.00. The Williams & Wilkins Co., Baltimore, Md.

Polarography in Medicine, Biochemistry, and Pharmacy. By M. BREZINA AND P. ZUMAN. With a Foreword by J. Heyrovsky. Revised English Edition Translated from the Czech by S. Wawzonek. 862 pp., $19.50. Interscience Publishers, New York, N. Y.

Poliomyelitis. Papers and Discussions Presented at the Fourth International Poliomyelitis Conference. 684 pp., $7.50. J. B. Lippincott Co., Philadelphia, Pa.

Polysaccharides in Biology. Transactions of the Third Conference May, 1957. Edited by GEORG F. SPRINGER. 249 pp., $4.75. The Josiah Macy, Jr. Foundation, New York, N. Y.

Practical Leads to Puzzling Diagnoses. Neuroses that Run through Families. By WALTER C. ALVAREZ. 490 pp., $9.00. J. B. Lippincott Co., Philadelphia, Pa.

Preventive Medicine in World War II. Vol. IV. Communicable Diseases. Prepared and published under the direction of Maj. Gen. S. B. Hays, Surgeon General. Edited by Col. John Boyd Coates, Jr., assisted by Ebbe Curtis Hoff and Phebe M. Hoff. 544 pp., $5.50. Superintendent of Documents, U. S. Government Printing Office, Washington, D. C.

CONNECTIVE TISSUE STUDIES[1]

I. Relation of Dietary and Tissue Levels of Ascorbic Acid to the Healing of Surgically-induced Wounds in Guinea Pigs

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The relation of nutrition to the chemistry of wound healing is of outstanding interest, and of special importance in this problem is the part played by ascorbic acid. In most reports on the chemistry of wound healing in guinea pigs, diets of various composition were used (1a–d). Since the adequacy of such diets is uncertain, it seemed important to us to make studies of wound healing uncomplicated by other dietary deficiencies. Slack (2) has recently reported such difficulties. For this reason the semi-synthetic diet of Reid and Briggs (3) was chosen. This diet seemed advantageous in that it has a high protein, mineral and vitamin content, and contains all dietary constituents essential for the guinea pig. Young guinea pigs upon this diet showed an average weight gain of 7.1 gm. per day, adequate blood cell formation and normal reproduction.

It has been observed by Penney and Zilva (4) and recently noted by Collins and Elvehjem (5) that oral doses of ascorbic acid above a certain level are only partially absorbed by the guinea pig. Another advantage of the semi-synthetic diet, which can be prepared in a powder form, is that ascorbic acid supplement can be thoroughly mixed in the diet so that there is very little interference with absorption. In all the experiments on which animals were fed commercial pellet type of diet, it was necessary to administer the total daily requirement of ascorbic acid by dropper. When simultaneous experiments using pellet and semi-synthetic diets were being carried out, the entire daily ascorbic acid requirement was administered in both instances by dropper and resulted in poor absorption of vitamin C. Hou (6) noted that double the minimal protective dose of ascorbic acid was required when the vitamin was administered orally, compared with subcutaneous injection. This partial absorption of ascorbic acid above certain levels is peculiar to the guinea pig and does not occur in the human (7a–b).

In this paper we are presenting a study in guinea pigs of the relation of the dietary intake of ascorbic acid to the concentration of ascorbic acid in the blood, adrenal glands, and in the abdominal skin and muscle of the scar and distant tissue. The pressure needed to rupture surgically-induced wounds in relation to ascorbic acid intake has been noted as well as comparative study

[1] Aided by NIH U. S. Public Health Service Grant A-1949.

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of Purina rabbit chow diet and the semi-synthetic diet of Reid and Briggs (3). To whatever extent such information is transferrable, it is of interest clinically, since in human patients the dietary intake of vitamin C is probably related to the efficiency of wound healing as well as to the concentrations of ascorbic acid in the healing wound and the level in the blood and tissues (8a–c).

MATERIALS AND METHODS

Young male guinea pigs of the Hartley strain, weighing 220–250 gm., were used. Originally, attempts were made to close the abdominal cavity with interrupted wire sutures (9). A midline abdominal incision according to the operative technique of Bartlett, Jones and Ryan (1d) proved more satisfactory. To measure the strength of the healing wound in the abdominal wall, the animal was anaesthetized with nembutal and the pressure at which the wound broke open was determined by inflating the peritoneal cavity with air until rupture occurred. The wounds ruptured in two layers, the skin scar first and then the muscle scar. The pressure at the time of rupture of the scar layers was recorded in mm. of Hg. with a sphygmomanometer connected by needle and rubber tubing with the peritoneal cavity. The sphygmomanometer was calibrated from 0 to 700 mm. of Hg. We preferred this method for testing wound strength to tensile strength measurements (10). Blood samples and tissues were taken for analysis immediately after the measurements of the pressure of wound rupture. The abdominal skin and muscle tissues appear in the tables as "skin and muscle scar", denoting actual scar tissue, and "distant abdominal skin and muscle" taken at a distance of 3 cm. from the line of the abdominal incision. The ascorbic acid content of blood and tissues was determined by the method of Roe and Kuether (11).

EXPERIMENTAL PROCEDURE AND RESULTS

In Table I are shown the results of a study of two types of diet, Purina rabbit chow, and the semi-synthetic diet of Reid and Briggs (3). Male guinea pigs of comparable weights from the same colony were divided into two groups and kept under identical housing conditions. After having been fed these diets with an ascorbic acid intake of 60 mg. per pig per day by dropper for a period of 10 days, abdominal operation was performed as described. At the time of operation samples of skin and muscle were taken from the area of the laparotomy and a sample of blood was collected by the method of Vallejo-Freire (12). The animals were fed their respective diets with the 60 mg. per day supplement of ascorbic acid by dropper for 10 additional days and then sacrificed. Anaesthesia was induced with nembutal, the pressures at which the wounds ruptured were recorded in millimeters of mercury. The wounds ruptured in two stages, a separation of skin occurred first followed by rupture of the muscle layer at higher pressures. Blood samples were obtained, and samples of adrenals, skin scar and distant abdominal skin, muscle scar and distant abdominal muscle were removed and analyzed for ascorbic acid. The weights of the animals at operation and at sacrifice were recorded.

Significant differences between the ascorbic acid contents of the blood and tissues of the animals upon the two diets were not observed. As shown in

TABLE I

Comparison of Diets

Diet	Weight, gm.		At Time of Operation Ascorbic Acid, mg. per 100 ml. or gm.			At Time of Sacrifice							
						Pressure for Wound Rupture, mm. Hg.		mg. Ascorbic Acid per 100 ml. or gm.					
	Operation	Sacrifice	Blood	Abdominal skin	Abdominal muscle	Skin	Muscle	Blood	Skin scar	Distant abdominal skin	Muscle scar	Distant abdominal muscle	Adrenals
Purina chow	374	408	1.27 ±.12	9.9 ±.3	4.7 ±.8	258 ±8	280 ±21	.36 ±.05	13.3 ±.9	7.5 ±.4	7.0 ±.5	2.8 ±.2	108 ±.8
Reid-Briggs	322	335	1.36 ±.14	11.9 ±.3	5.0 ±.3	236 ±5	280 ±10	.35 ±.03	11.7 ±.5	7.9 ±.3	5.3 ±.4	2.5 ±.1	84 ±4.9

Comparison of diets. Effect of Purina chow and Reid-Briggs Diet (No. 13) on tissue levels of ascorbic acid and the pressures at which wound rupture occurs. There were 5 male guinea pigs on each diet. Each pig received 60 mg. of ascorbic acid per day by dropper for 10 days before operation and 60 mg. per day during the period between operation and sacrifice. The ± designations indicate the standard error.

Table I, the pressures necessary for wound rupture did not vary significantly between the groups on the two diets. The pigs on the Purina chow showed a somewhat greater weight gain.

Since a high level of ascorbic acid was fed in these experiments, a comparison of the adequacy of the two diets in nutritional qualities may be made. In all of the factors examined the Purina chow gave as good a response as the semi-synthetic diet of Reid and Briggs (3).

The data of Table II show the effect of different dietary intakes of ascorbic acid on wound healing and tissue levels. The pressures required for rupture of the healing wound were significantly lower in the scorbutic groups. In the groups receiving ascorbic acid supplements the wound rupture pressures correlated roughly with the ascorbic acid intakes. As shown by Figure 1, there was a good correlation between the dietary intake and blood and tissue levels of ascorbic acid.

An observation of interest in Table I was the decrease in the blood level of ascorbic acid following operation. At operation the average whole blood levels per 100 ml. were 1.27 mg. and 1.36 mg. on Purina chow and the Reid-Briggs diet, respectively. Even though the intake of ascorbic acid was 60 mg. per day, the blood levels decreased to 0.36 mg. and 0.35 mg. per 100 ml. at the time of sacrifice.

This drop in blood level following operation prompted us to note the level of ascorbic acid in the blood, abdominal skin and muscle of unoperated animals in order to evaluate the effect of operation or stress thus created as a causative

TABLE II

Effect of Dietary Ascorbic Acid

Group No.	Ascorbic Acid Intake Per Day, mg.	At Time of Operation Ascorbic Acid, mg. per 100 ml. or gm.			At Time of Sacrifice							
					Pressure for Wound Rupture, mm. Hg.		mg. Ascorbic Acid per 100 ml. or gm.					
		Blood	Abdominal skin	Abdominal muscle	Skin	Muscle	Blood	Skin scar	Distant abdominal skin	Muscle scar	Distant abdominal muscle	Adrenals
1	0	0.21	4.7	2.1	100	119	—	—	—	—	—	—
		±.01	±.7	±.1	±9	±11						
2	0	—	7.4	4.9	188	190	.12	2.9	—	1.6	—	13.7
			±.9	±.6	±10	±10	±.01	±.4		±.04		±.4
3	0	0.6	8.2	2.4	176	178	.09	2.5	2.4	0.8	.9	6.5
		±.04	±2	±.2	±11	±11	±.01	±.3	±.7	±.1	±.1	±.9
4	0	—	7.7	2.7	161	161	.09	0.9	—	0.5	.47	4.2
			±.8	±.5	±11	±11	±.01	±.3		±.1	±.15	±.2
5	6	.35	5.4	2.7	215	278	.16	5.5	4.1	3.0	1.7	32.7
		±.07	±.6	±.3	±7	±8	±.04	±.9	±.4	±.5	±.2	±6
6	30	1.40	12.1	6.6	230	247	.68	17.0	10.5	9.7	4.6	132
		±.07	±.9	±.2	±21	±17	±.06	±2	±2	±.8	±.6	±5
7	90	1.83	12.1	5.9	272	305	1.08	17.5	10.8	9.3	4.3	140
		±.09	±1	±.3	±6	±6	±.09	±1	±1	±1	±.6	±6

Effect of different dietary intakes of ascorbic acid on blood and tissue levels of ascorbic acid and on the pressure required for rupture of wounds in operated male guinea pigs. The diet of Reid and Briggs (No. 13) was used except for the third and fourth scorbutic groups which were fed Purina rabbit chow. All groups were upon the diet for 10 days before operation and 10 days between operation and sacrifice. The ± designations indicate the standard error.

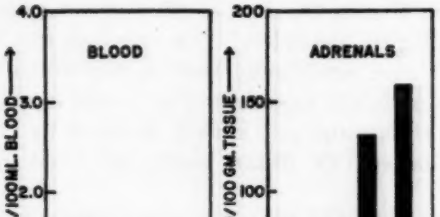

FIG. 1. Relation of varying levels of dietary intake of ascorbic acid to its concentration in blood and adrenals.

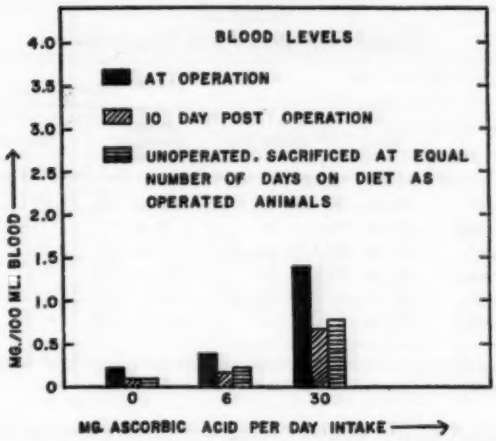

FIG. 2. Blood levels in unoperated animals compared with levels in operated animals on different dietary intake.

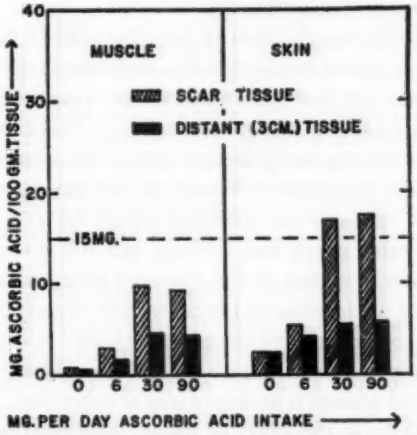

FIG. 3. Relation of varying levels of dietary intake of ascorbic acid to its concentration in skin and muscle of wound scar tissue and skin and muscle of distant abdominal tissue.

factor for this decline in values. In Figure 2 it will be noted that the blood level in the unoperated animal is about the same as in the operated animal at various dietary intakes at the same time interval.

A significant finding noted in Table II is the higher level of ascorbic acid in the skin and muscle scar as compared with the level in distant abdominal skin and muscle at the time of sacrifice. The same difference between concentrations of ascorbic acid in the skin and muscle of scar and distant abdominal tissues is seen in Table I. Figure 3 shows that at the scorbutic level no concentration of

TABLE III

Comparison of Ascorbic Acid Tissue Levels

Ascorbic Acid Intake Per Day		At Sacrifice				
		Blood	Muscle Scar	Distant Abdominal Muscle	Skin Scar	Distant Abdominal Skin
0 mg.	Operated	.09 ± .01	.87 ± .16	.84 ± .13	2.50 ± .29	2.46 ± .72
	Unoperated	.09 ± .01	—	.9 ± .1	—	1.9 ± .3
6 mg.	Operated	.16 ± .04	3.04 ± .55	1.72 ± .2	5.54 ± .94	4.14 ± .42
	Unoperated	.19 ± .04	—	1.67 ± .17	—	5.08 ± .67
30 mg.	Operated	.68 ± .06	9.8 ± .83	4.6 ± .58	17.03 ± 2.38	10.5 ± 1.9
	Unoperated	.77 ± .08	—	4.8 ± .22	—	10.5 ± .52

Comparison of tissue levels in operated and unoperated animals after 20 days on diets of varying ascorbic acid intake. The ± designations indicate the standard error.

ascorbic acid occurs in the wound tissue, but the level of ascorbic acid in the healing wound rises and the difference in levels between scar and distant tissues becomes more apparent with increased dietary intake. A leveling of concentration occurs in the higher dietary intakes.

In contrast to the increased levels of ascorbic acid in the wound tissue at sacrifice, the level of ascorbic acid in the skin and muscle of unoperated animals at the same time interval was equivalent to the values found in the distant abdominal skin and muscle of the operated group. This can be noted in Table III. For example, on a 6 mg. daily dietary intake the ascorbic acid level of the skin in the unoperated group was 5.08 mg. per 100 gm. tissue, as compared to 4.1 mg. per 100 gm. tissue in the operated group; the level in the abdominal muscle of the unoperated group was 1.67 mg. per 100 gm. tissue, as compared to the distant abdominal muscle of the operated group which was 1.7 mg. per 100 gm. tissue. Thus, the increase in concentration of ascorbic acid in the healing wound tissue as compared with the concentrations in the abdominal tissues of the unoperated animal as well as with the abdominal tissues distant from the wound in the operated animal is an important observation.

A more critical study of the effect of tissue concentrations of ascorbic acid at shorter intervals between the times of operation and sacrifice is shown in Table IV. In these experiments data were obtained at 3 day intervals for 12 days. For a preparatory period of 8 days the animals were placed on the semi-synthetic diet of Reid and Briggs (3), supplemented with 2 mg. of ascorbic acid per gram mixed in the food. Each animal thus received approximately 60 mg. of ascorbic acid per day, an intake that saturated the tissues with ascorbic acid. They were then divided into two groups; from the first group ascorbic acid was completely withdrawn, 90 mg. of ascorbic acid was added to the diet of the second group. After 10 days on their respective diets both groups were operated, and sacrificed 3, 6, 9, and 12 days postoperatively.

TABLE IV

Relation of Wound Rupture to Ascorbic Acid

Group	At Time of Operation, mg. Ascorbic Acid per 100 gm.		Days After Operation	At Time of Sacrifice					
				Pressure for Wound Rupture, mm. Hg.		mg. Ascorbic Acid per 100 ml. or gm.			
	Abdominal skin	Abdominal muscle		Skin	Muscle	Blood	Skin scar	Muscle scar	Adrenals
Control	14.3 ±1.04	6.5 ±.83	3	174 ±18.65	180 ±11.88	1.85 ±.26	13.0 ±.76	10.5 ±.28	182 ±2.70
Scorbutic	10.7 ±.86	4.9 ±.72	3	144 ±10.80	155 ±7.76	0.30 ±.10	2.3 ±.49	1.3 ±.17	16.2 ±4.71
Control	9.5 ±.72	5.4 ±.73	6	224 ±15.06	228 ±9.60	1.26 ±.17	12.5 ±.97	7.9 ±.63	160 ±5.56
Scorbutic	6.9 ±.45	3.6 ±.49	6	166 ±20.54	174 ±11.57	.17 ±.01	2.3 ±.15	1.3 ±.26	29.8 ±7.40
Control	12.1 ±1.10	5.9 ±.35	9	272 ±6.60	305 ±5.50	1.08 ±.10	17.5 ±1.08	9.3 ±1.17	179 ±7.98
Scorbutic	7.4 ±.96	4.9 ±.65	9	188 ±10.36	190 ±4.82	.12 ±.01	2.9 ±.45	1.6 ±.42	13.7 ±4.08
Control	15.1 ±.94	7.7 ±.84	12	254 ±11.60	284 ±7.50	.72 ±.04	16.0 ±1.44	8.9 ±.55	151 ±5.96
Scorbutic	11.7 ±1.17	4.6 ±1.25	12	210 ±8.07	220 ±6.73	.16 ±.02	2.8 ± .43	1.6 ±.51	22.3 ±7.85

Relation of pressure required for wound rupture to dietary intake and tissue levels of ascorbic acid. There were 5 guinea pigs in each group. For a preparatory period of 8 days all animals received 60 mg. of ascorbic acid daily for saturation. Thereafter, ascorbic acid was completely withdrawn from the scorbutic group, and 90 mg. ascorbic acid per animal was supplemented to diets of remainder. After 10 days, animals were operated and sacrificed at 3, 6, 9, and 12 days postoperatively. The ± designations indicate the standard error.

As noted in Table IV, a highly significant difference ($P < .01$) between control and scorbutic groups occurred at the 3, 6, 9, and 12 day intervals after operation when ascorbic acid values for blood, adrenals, skin scar, and muscle scar were compared. The data on the pressures required for wound rupture showed a steady increase at each interval up to the 12th day in both the control and the scorbutic groups. A lower average value for the pressure measurements was observed in the scorbutic group in all comparisons. At the 3 day postoperative interval the wound rupture pressures for the control and scorbutic groups did not vary significantly ($P = 0.2$). At the 6th day interval the variance between the control and scorbutic groups was significant ($P = 0.03$).

It is of particular interest that the pressure of rupture of the wounds in the scorbutic animals increased steadily throughout the 12 day period. Ascorbic acid in the skin and muscle scar was found by the analyses to be low, and remained at this low level through the 12 day postoperative period, a total of 22 days on the scurvy diet. It is noted in Figure 4 that the increment of increase

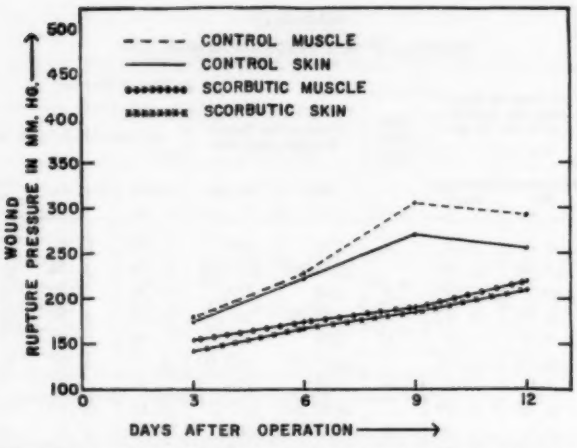

FIG. 4. Pressure of wound rupture of skin and muscle of control and scorbutic animals in mm. of Hg. at 3, 6, 9 and 12 days after operation. Control group 90 mg. dietary ascorbic acid daily. Scorbutic group 0 mg. ascorbic acid in diet.

of resistance to rupture of the wound was much smaller in the scorbutic animals than in the control animals that received an adequate ascorbic acid intake.

DISCUSSION

The technique used for measuring the pressure required for wound rupture is obviously a gross measurement. However, there was a proportional relationship between the dietary intake and tissue levels of ascorbic acid, on the one hand, and the pressure of rupture of the healing wound, on the other. Our results confirm and extend earlier studies (1).

It is noted from our data that there is an excellent correlation between ascorbic acid blood and tissue levels to varying levels of dietary intake. A decrease in the blood level of ascorbic acid was noted following operation. This was shown to be independent of the operation from data which we obtained on unoperated guinea pigs. The fact that all of our animals were young and in the growth period and therefore probably had a greater demand for ascorbic acid because of growth, may account for this decrease in levels. Elman (13) has pointed out that abdominal and other incisions in older individuals healed quite as rapidly as do those in younger individuals. At a future date we intend to perform the same measurements on adult animals.

In a study of human patients, Crandon *et al.* (14) note that Lund (15) in 1939 reported a fall in the blood level of ascorbic acid following operation from the original level. However, the differences in blood levels as reported by

Crandon *et al.* (14) were nominal, and they noted no correlation between the degree of drop in blood ascorbic acid and the duration or type of either surgery performed or anaesthesia administered. However, Crandon *et al.* (14) make the important observation that over one-third of patients suffering from wound dehiscence were suffering serious dietary ascorbic acid deficiency as indicated by their blood levels.

In the comparative study of the Purina rabbit chow and the Reid-Briggs diet (3) the pigs on the Purina chow showed a slightly greater average weight gain, probably because the food consumption of the chow was greater. The protein content of Purina chow is 15 per cent and that of the Reid-Briggs diet (3) is 30 per cent. The protein in the Reid-Briggs diet (3) is casein, which has a good methionine content.

The exact role of protein in wound healing is at present disputed and uncertain (16a–c). It is however, evident from our data that the Purina chow containing the lower amount of protein is adequate. The fact still remains that protein deficiency may predispose to wound infection and thus give rise to disruption of the wound (17).

The observation that ascorbic acid is present in an increased concentration in the healing wound is a significant finding. We are continuing our studies, employing radioactive ascorbic acid, to learn the source and the metabolic significance of this increase. That there is an increased need for ascorbic acid following surgery is generally accepted, and the data here presented substantiates the rationale for the clinical pre- and postoperative administration of optimal levels of ascorbic acid.

SUMMARY

1. Studies have been made of the effect of ascorbic acid upon the efficiency of the repair process in surgically-induced abdominal wounds in guinea pigs.

2. Comparison of Purina rabbit chow and the Reid-Briggs semisynthetic diet showed a remarkably similar response with respect to the pressure of rupture of the healing wound, blood and tissue levels of ascorbic acid when the vitamin was fed at a level of 60 mg. per day.

3. There was a rough correlation between the dietary intake of ascorbic acid and the pressure of rupture of the healing wound, while there was a close correlation between the ascorbic acid intake and the blood and tissue levels.

4. With increasing levels of ascorbic acid intake, its concentration in the wound tissue rose above the concentration in distant tissue. At supersaturation intake levels, there was no further increase in the tissue.

5. Tissue levels of unoperated animals were determined and found to correspond to the levels of ascorbic acid in the distant abdominal tissues of the operated animals. This would seem to indicate that the operation *per se* or various stresses contingent upon the operation are not causative factors in

delaying wound healing or lowering ascorbic acid concentrations in blood or tissues.

6. Even in the scorbutic animal a certain degree of wound healing occurs, though the level of increment of increase of healing in the scorbutic is considerably lower than in the animal on optimal ascorbic acid intake.

7. The data obtained from these animal experiments on wound healing substantiate the need for optimal dietary intake of ascorbic acid in the clinical pre- and postoperative patient.

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STRETCH EFFECTS IN HUMAN EXTRAOCULAR MUSCLE

An Electromyographic Study[1, 2]

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Although the initial observations of Sherrington (15) concerning supranuclear control of eye movements is accepted, the role played by proprioception is less clear. It is generally agreed that conscious positional sense is not subserved by receptors in eye muscles (10, 11). The role of myotatic mechanisms, however, in governing contraction of the extraocular muscles is less well documented and may vary amongst different species. In the goat, typical muscle spindles (4) and afferent impulses in IIIrd cranial nerves resulting from muscle pull have been demonstrated by Cooper, Daniel and Whitteridge (8). On the contrary, in cat and rhesus monkey, muscle spindles have not been observed and whereas Cooper *et al.* (6, 7) have recorded afferent discharges to stretch, McCouch and Adler (13) and McIntyre (14) have failed to demonstrate these responses. In humans, muscle spindles have been observed by Cooper and Daniel (5) and one might therefore anticipate the presence of tension recording mechanisms. The purpose of the present study was to investigate, by electromyographic means, the possible existence of such stretch mechanisms in human extraocular muscles.

METHODS

Eight patients were investigated. The ophthalmic diagnoses in 7 are listed in the Tables. The studies in these patients, all of whom had full ocular movements, were made prior to enucleation of one eye. In three of these, unilateral retrobulbar alcohol injection (12) had been performed several months previously. In the remaining patient, a recent case of IIIrd nerve palsy, the muscle remained *in situ* and stretch was applied through a forceps placed at the insertion. A similar method of stretch was employed in the normally innervated eyes of those 3 patients with retrobulbar injection.

Under topical anesthesia (Ophthaine)[3], a concentric needle electrode was inserted into one of the horizontal recti muscles. In those patients studied prior to enucleation, additional stability of electrode position was achieved by suturing the needle in place. Muscle action potentials were then led to amplifiers, displayed on the beams of a cathode ray oscilloscope, and photographed. Recordings were made with the eyes both in primary and in horizontal gaze, the latter about 30 degrees beyond the midline. When enucleation was to follow, a suture was placed through the tendon of one horizontal rectus muscle which was then dis-

[1] Part of this material was presented to the Wilmer Residents' Meeting in March, 1958.

[2] Supported by a grant (B-367) from the National Institute of Neurological Diseases and Blindness, U. S. Public Health Service.

[3] E. R. Squibb & Sons, New York.

TABLE I

Stretch Effects on Agonist Contraction

Diagnosis	Stretched Muscle		
	Agonist	Antagonist	Yoke
1. Traumatic retinal detachment	+	+	
2. Penetrating injury with glaucoma	+	+	
3. Concussion glaucoma	0	0	0
4. Traumatic dislocation of lens with glaucoma	+	+	

+, Inhibitory effect; 0, no effect.

TABLE II

Stretch Effects on Agonist Contraction

(Unilateral Retrobulbar Alcohol Injection)

Diagnosis	A, Normal Eye Stretched Muscle			B, Injected Eye Stretched Muscle		
	Agonist	Antagonist	Yoke	Agonist	Antagonist	Yoke
5. Retinal vein occlusion with glaucoma	+	+		0	0	0
6. Intraocular foreign body with glaucoma	+	+		0	0	
7. Traumatic dislocation of lens with glaucoma			+	0	0	0

+, Inhibitory effect; 0, no effect.

inserted distal to this ligature. The effects of this disinsertion, and of gentle manual pull on the suture, when the muscle was acting as agonist, antagonist or yoke[4] were assessed by changes in amplitude and frequency of action potentials in the agonist muscle. In all instances, the direction of pull was parallel to the muscle fibers, and care was taken not to displace the eye.

RESULTS

Action potentials from intact horizontal extraocular muscles in primary and lateral gaze were similar in amplitude and frequency to those previously reported by Björk and Kugelberg (1) and Breinin and Moldaver (2).

I. *Primary Gaze.* With the eyes in primary position, neither disinsertion nor muscle stretch had any detectable effect on the amplitude or frequency of action potentials recorded from the horizontal recti.

II. *Agonist Contraction.*

a. *Normal innervation.* Action potentials from the contracting agonist were not altered by disinsertion of this muscle or its antagonist. Certain alterations were, however, introduced by muscle stretch. These are summarized in Tables I and IIA. In 5 of 6 patients studied prior to enucleation, passive stretch either

[4] The terms "agonist", "antagonist" and "yoke" refer solely to the direction of voluntary gaze. Thus, in right lateral gaze, for example, the right lateral rectus is the agonist, right medial rectus is the antagonist, and left medial rectus is the yoke muscle.

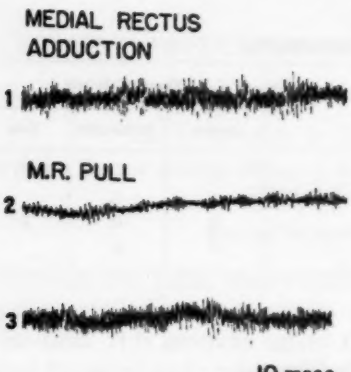

FIG. 1

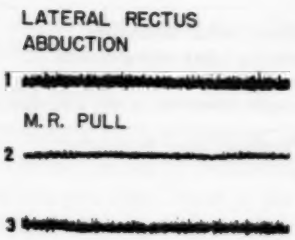

FIG. 2

FIG. 1. The effect of passive agonist stretch on agonist contraction. See text.
FIG. 2. The effect of passive antagonist stretch on agonist contraction. See text.

of agonist or its antagonist during voluntary contraction produced a decrease in amplitude and frequency of action potentials recorded from the agonist muscle. In Figure 1, for example, recordings were made from a medial rectus muscle during agonist contraction. Tracing 2 of that figure reveals a decline in frequency and amplitude of the action potentials when the insertion of this muscle was passively stretched. With the release of stretch in tracing 3, the muscle action potentials returned to their previous activity. In Figure 2, the effect of antagonist stretch on agonist contraction is demonstrated. A similar decrease in amplitude and frequency of the action potentials in the agonist occurred (Tracing 2).

In 2 cases of 3, stretch of the yoke muscle effected a decrease in the motor discharge from the contracting agonist of the opposite eye.

b. *Retrobulbar alcohol injection.* In these patients action potentials recorded from the extraocular muscles of the injected eye were completely normal. In none of these previously injected eyes, however, was amplitude or frequency of the action potentials during agonist contraction diminished, when agonist or antagonist was passively stretched (Table IIB). Inhibitory effects were obtained, however, in the intact eye with passive stretch of the ipsilateral agonist and antagonist muscles. In addition, in patient No. 7, stretch of the normally innervated yoke muscle produced a decrease in the action potentials recorded from the contralateral agonist.

c. *Third nerve palsy.* No electrical activity was recorded during attempts at

volitional contraction in the field of action of the involved muscles. Normal action potentials were obtained, however, from lateral rectus and these were not altered when the paretic medial rectus was pulled.

DISCUSSION

In primary gaze, neither disinsertion nor stretch produced any change in the configuration of the action potentials recorded from the same muscle. Release of muscle from its insertion alone, however, does not permit complete retraction. This fact could account for the difference between the findings reported here and those of Breinin (3). In his postenucleation studies electrical silence developed following *complete* muscle retraction.

During agonist contraction, on the other hand, manual pull of agonist itself or its antagonist exerted a demonstrable inhibitory influence on the agonist action potentials. Indeed this was the only effect and it was noted in 5 of 6 normally innervated eyes. Mechanical stimulation of stretch receptors within the extraocular muscles might be the cause of this inhibition. In support of this thesis, the results in the 3 patients with previous retrobulbar alcohol injection are of considerable interest. In these patients, action potentials in the contracting agonists were unchanged by stretch of any muscle in the injected eye. On the contrary, muscle stretch of the contralateral normal eye resulted in inhibition. Certainly some sensory neurones from the eye had been destroyed by those injections, as judged by a definite depression of corneal sensation in all three patients. The absence of inhibition, therefore, might well have been caused by similar damage to afferent fibers from the muscles. This cannot yet be substantiated histologically. In one of these muscles, however, Dr. J. Reimer Wolter of Ann Arbor, Michigan, was unable to delineate intact sensory endings despite relative preservation of motor end plates. The fact that no inhibitory effects were obtained from the patient with IIIrd nerve palsy when a paretic muscle was stretched, also suggests involvement of afferent neurones.

These inhibitory effects vary markedly from the responses both to phasic and tonic stretch of skeletal muscle (9). This may well have a ready explanation in the histological and physiological differences between extraocular and skeletal muscles (5, 9). Certainly an inhibitory myotatic mechanism must play a very limited role in controlling ocular movements. On the other hand, inhibition may influence motor unit discharge so that alternating groups of muscle fibers participate in the performance of a coordinated movement.

Pain resulting from pull of extraocular muscles is a common clinical experience. Although these experiments were performed under local anesthesia, some patients did experience more pain than others. In those patients, however, in whom pain was a prominent feature, the inhibitory effect of muscle stretch was less apparent and in 1 case absent.

Electrode movement, during passive stretch, was minimized in 7 patients

by suturing the electrode in place. It might be suggested that decreased action potentials, interpreted as inhibition, resulted from mechanical displacement. This is unlikely, however, for two reasons. Firstly, similar inhibition occurred with antagonist pull. More convincing, however, was the complete absence of inhibition in the eyes with previous retrobulbar injections.

SUMMARY

In humans, muscle action potentials were recorded in primary and lateral gazes from one horizontal rectus muscle, and the effect of passive stretch was observed. With the eyes in primary gaze, the action potentials were unchanged by disinsertion or manual pull. During agonist contraction, however, stretch of the agonist itself, the antagonist or yoke muscle produced a decrease in the frequency and amplitude of the discharges. This inhibitory effect was not observed when stretch was applied to the muscles of an eye which had a previous retrobulbar alcohol injection or the involved muscle in a patient with a IIIrd nerve palsy. These results suggest that the extraocular muscles of man do have receptors which can be stimulated by passive stretch.

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THE CYTOCHEMICAL LOCALIZATION OF OXIDATIVE ENZYMATIC ACTIVITY AND GLYCOGEN IN FROG STRIATED MUSCLE[1]

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Recently Nachlas, Walker and Seligman (1, 2) reported histochemical methods which are capable of specifically demonstrating the site of diphosphopyridine nucleotide (DPN) and triphosphopyridine nucleotide (TPN) diaphorase and certain of the DPN linked and TPN linked dehydrogenases. Nachlas, Tsou, deSouza, Cheng and Seligman (3) have also provided a histochemical method for the demonstration of succinic dehydrogenase activity. These methods employ the ditetrazole, 2,2′-di-(para-nitrophenyl)5,5′-diphenyl-3,3′(3,3′ dimethoxy-4,4′ biphenylene) tetrazolium chloride or simply nitro-BT which has been shown by Tsou, Cheng, Nachlas, and Seligman (4) to possess favorable properties for cytochemical studies. In addition, Nachlas, Crawford, Goldstein and Seligman (5) have developed a reliable method for the demonstration of cytochrome oxidase. Furthermore, the reliability of the periodic acid-Schiff method (6) when conducted with an appropriate amylase treated control permits a study of the cytochemical localization of the substrate glycogen.

Although skeletal and cardiac muscle have been studied histochemically by several investigators (4, 7, 8, 9 and 10) the enzyme localization described has not extended beyond muscle mitochondria. Wachstein and Meisel (7) reported that a diffuse staining of cross striations was seen occasionally only in human post-mortem striated muscle and it was suggested that this represented a non-specific adsorption of reduced dye. Apparently no experimental controls were employed in this study to clarify or substantiate this interpretation. Calcutt (11) reported the use of several tetrazolium salts for the supra-vital staining of mouse striated muscle. In this brief note, the reduced formazan was seen to be concentrated in the cross bands. Although Calcutt does not identify the cross banding his description and photomicrographs indicate that the A and Z bands were stained. This author states that the distinctive distribution of reduced formazan "is strongly suggestive of a similar distribution of reducing groups within the muscle cell."

Recently, Scarpelli, Hess, and Pearse (12, 13) described a modification of

[1] Aided by a grant from the National Foundation. The authors are indebted to Professor David Bodian for the interest shown during the progress of this study. In addition, the technical assistance of Mr. V. Talbert, Mrs. V. Prinn, and Mrs. N. Taylor is acknowledged.

the techniques employed in this study. Instead of nitro-BT, the method uses a N-thiazol-2-yl monotetrazolium chloride as an indicator of enzymatic activity. These authors obtained essentially the same results as Nachlas, *et al.*, (1, 2). It would appear that the experiments of Scarpelli, *et al.*, confirm the usefulness of these methods for the cytochemical localization of individual oxidative enzymes.

The object of the present report is twofold: 1. To provide more specific information regarding the cytochemical localization of glycogen and of several oxidative enzymes in glycerol extracted and intact (normal) skeletal muscle and 2. to develop a hypothesis concerning the morpho-physiological mechanism of internal activation of contraction and the metabolic activity in muscle cells in which both oxidative enzymatic systems and the transverse myofibrillar bridges would be expected to play important roles.

MATERIALS AND METHODS

The sartorius muscles of freshly killed frogs, kept at resting length, were quickly frozen by immersion in isopentane which was maintained at −70°C. with a mixture of acetone and dry ice. In addition, other sartorius muscles were extracted first in 50 per cent glycerol at −15°C. for 8 days and thereafter for an additional 4 days in 25 per cent glycerol at 0°C. The glycerol solutions were changed twice daily. Two hours before preparing frozen sections the tissue was transferred to distilled water at 0°C. The extracted muscle was then frozen by the method described above. Sections were cut (1 to 4μ) in the cryostat using a rotary microtome, mounted on coverslips, placed in incubation solutions, and incubated aerobically at 37°C. for 10 minutes, 30 minutes, 60 minutes, and at 27°C. overnight. The sections were fixed in formalin for 10 minutes, and either mounted in glycerol gel or transferred through a series of graded alcohols, absolute acetone, xylol, and finally mounted in permount.

Incubation Solutions.

The incubation solutions were prepared exactly as recommended by Nachlas, Walker, and Seligman (1, 2), Nachlas, Tsou, deSouza, Cheng and Seligman (3) and Nachlas, Crawford, Goldstein, and Seligman (5). These papers should be referred to for details.

The localization of each of the following enzymes was studied:

1). DPN diaphorase
2). Lactic dehydrogenase (DPN linked)
3). Malic dehydrogenase (DPN linked)
4). Isocitric dehydrogenase (DPN linked)
5). β-hydroxybutyric dehydrogenase (DPN linked)
6). TPN diaphorase
7). Isocitric dehydrogenase (TPN linked)
8). Malic dehydrogenase (TPN linked)
9). Glucose-6-phosphate dehydrogenase (TPN linked)
10). 6-Phosphogluconic dehydrogenase (TPN linked)
11). Succinic dehydrogenase
12). Cytochrome oxidase

Control sections were prepared by immersion in boiling water for 10 minutes followed by immersion in the various incubating media for an 8 hour period. To rule out the possibility of non-enzymatic tetrazole reduction, fresh sections were incubated in solutions containing

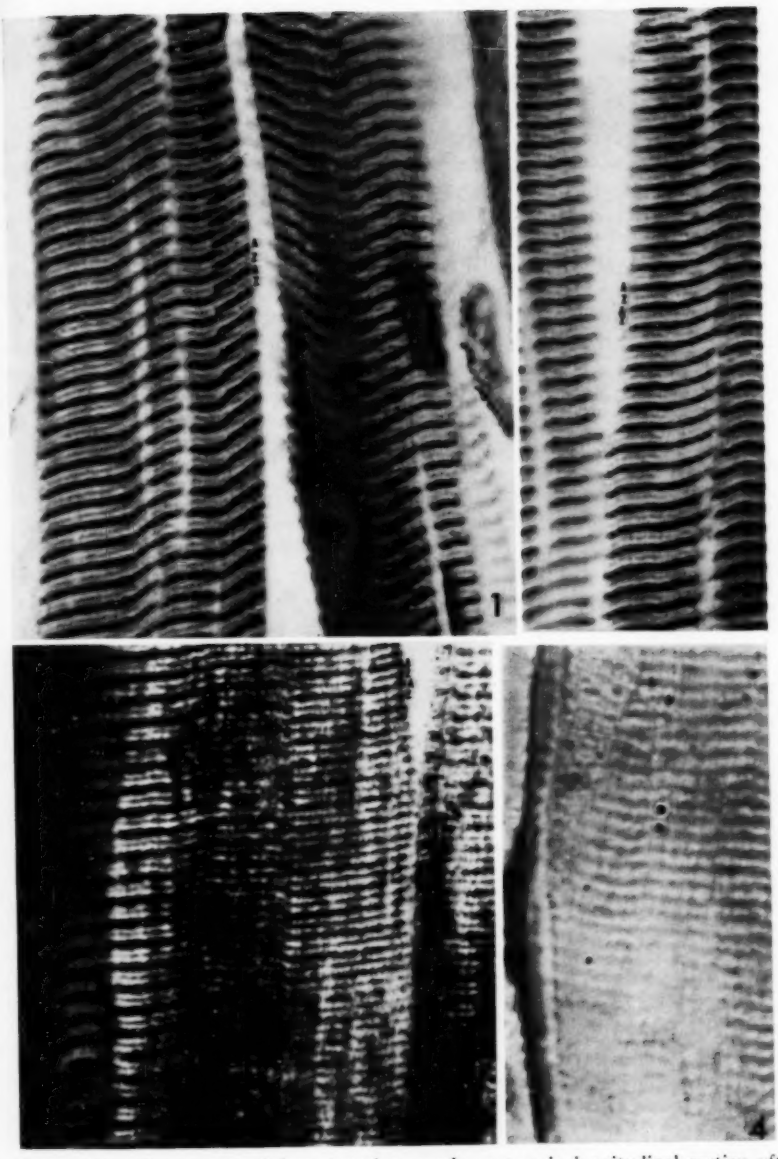


FIG. 1. Normal, unextracted frog sartorius muscle as seen in longitudinal section after silver staining. The bold black line (A) is the anisotropic band and the delicate black line (Z) is the Z disc and these represent the only argyrophilic components of the myofibrils. Bodian's protargol method. × 1500.

FIG. 2. Glycerol extracted frog sartorius muscle. The glycerol extraction (12 days duration) produced no discernible alteration in the banding. Bodian's protargol method. × 1500.

FIG. 3. Normal frog sartorius stained for glycogen. When this specimen is compared with that of Figure 1 it is seen that the PAS-positive bands correspond to those (A & Z) that are argyrophilic. Periodic acid-Schiff method. × 1500.

FIG. 4. The bands of the diastase treated control specimen obtained from the same ribbon as that shown in Figure 3 are colorless. The A and I bands are discernible only by virtue of their different refractive indices. Periodic acid-Schiff method. × 1500.

all of the reagents of the incubation mixture except the substrate. In addition, to eliminate the possibility that any of the results obtained may be due merely to the adsorption of formazan on to non-enzymatic areas, control sections were incubated for two hours in a buffered solution of the tetrazole. The latter was reduced by a commercial preparation of diaphorase prior to incubation with the frozen sections.

The PAS method employed for the demonstration of glycogen is that recommended by Lillie (6) and this method should be referred to for details. Frog sartorius muscles were fixed at resting length in absolute alcohol or in absolute alcohol saturated with picric acid. The fixed tissue was then transferred to xylol, then pine oil, and embedded in paraffin. Sections were cut at 5μ and mounted on glass slides. The muscle sections were deparaffinized in xylol and transferred through a hydrating series of alcohols to water and stained by the PAS method. Prior to PAS staining control sections were incubated for one hour at 37°C. in a 0.1 per cent solution of purified malt diastase (USP) buffered with Sorenson's phosphates (0.02M) at pH 6.0.

In addition to the cytochemical methods employed normal and glycerol extracted sartorius muscles were fixed at resting length in Bouin's fluid, 10 per cent formol-saline, absolute alcohol and picric acid-saturated absolute alcohol and stained by the Bodian protargol method (14). For control and comparative purposes, normal and glycerol extracted muscle specimens similar to those prepared for light microscopy studies were fixed in buffered (pH 7.2–7.4), isotonic 1 per cent osmium tetroxide and studied in an RCA EMU 3C electron microscope.

RESULTS

Glycogen (Fig. 3), DPN diaphorase (Fig. 5), DPN-linked dehydrogenases (Figs. 7, 9 and 10), succinic dehydrogenase and cytochrome oxidase (Fig. 11)

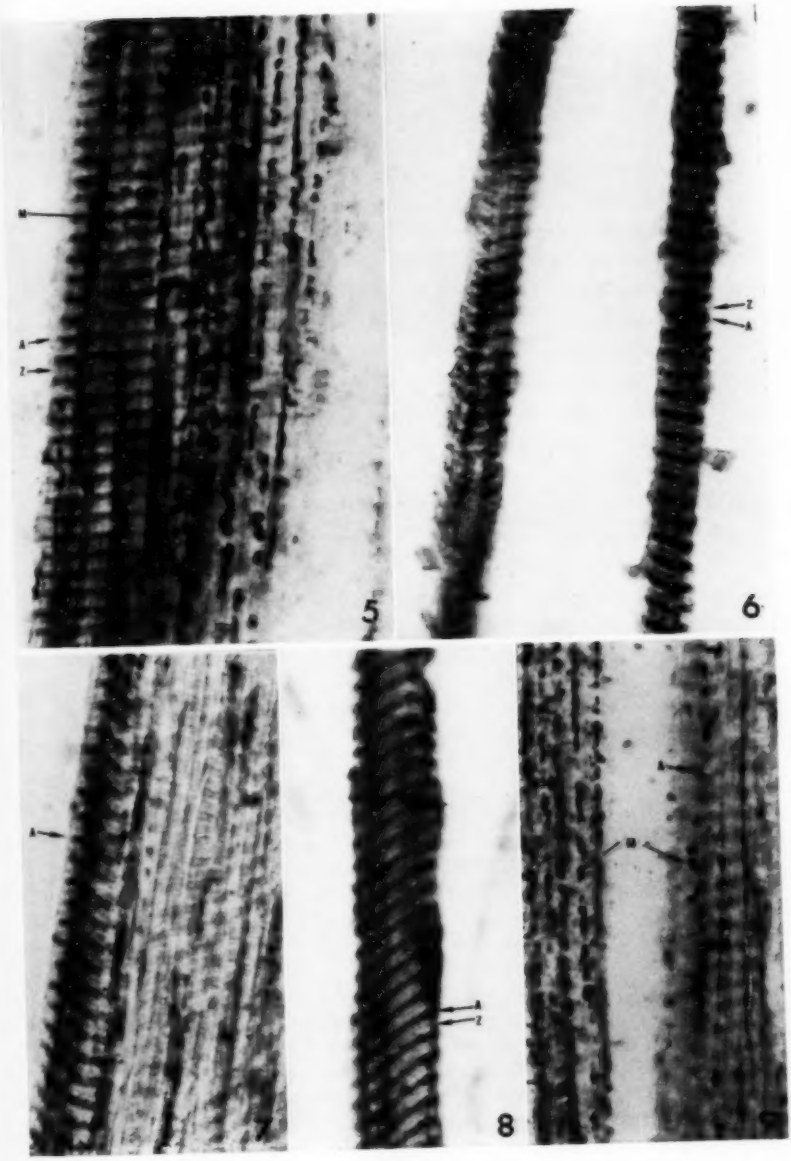
FIG. 5. Normal, unextracted frog sartorius stained for DPN diaphorase. Most intensely stained (blue in the actual specimen) are rod shaped bodies, presumably mitochondria, that are disposed end-to-end in chains, single file, in the interfibrillar spaces. The staining of the A bands is rather uniform and of moderate intensity. The Z lines are barely discernible. Nitro-BT method. 60 min. incubation. × 1500.

FIG. 6. Glycerol extracted sartorius stained for DPN diaphorase. After two hours incubation the A and Z bands attain a stain of maximal intensity. The cross banding is more discrete than that of the unextracted control (Fig. 5). Nitro-BT method. 2 hours incubation. × 1500.

FIG. 7. Normal, unextracted sartorius stained for DPN-linked malic dehydrogenase. Although the specimen, as a whole, was comparatively rich in malic dehydrogenase, in many fibers, randomly placed throughout the specimen, the stain was confined to the marginal zones as is illustrated in this instance. In the stained zone the A bands are more conspicuous than the mitochondria. Nitro-BT method. 10 min. incubation. × 1500.

FIG. 8. Glycerol extracted sartorius stained for malic dehydrogenase. The staining of the A and Z bands is identical to that representing DPN diaphorase as shown in Figure 6. Nitro-BT method. 2 hours incubation. × 1500.

FIG. 9. Normal, unextracted sartorius stained for DPN-linked isocitric dehydrogenase. This field was selected to illustrate the difference noted in adjacent fibers with respect to overall stain intensity and pattern of stain. The enzyme activity in the cell on the left is represented by the "over-stained" mitochondria while that on the right is represented mainly by the weakly stained A bands. Nitro-BT method. 60 min. incubation. × 1500.



FIGS. 5–9

were detected in the mitochondria and the myofibrillar Z and A bands of intact muscle. DPN diaphorase (Figs. 6 and 14) and malic dehydrogenase (Fig. 8) as well as the other enzymes studied were demonstrable in the Z discs and A bands of glycerol extracted preparations. TPN diaphorase activity was not demonstrable. The results of this study are summarized in Table I.

The distribution of glycogen in skeletal muscle was of particular interest since glycogen represents a major energy reserve in this tissue. In addition to its presence in the interfibrillar spaces a high content of glycogen was demonstrable in the Z and particularly A bands of the normal specimens of skeletal muscle (Fig. 3). This distribution of glycogen was consistent providing that the recommended fixatives and the PAS method were used. No PAS positive material remained in the muscle cells of specimens that had been previously exposed to diastase digestion (Fig. 4).

Aside from the particular enzymes demonstrated and the organelles showing activity the most noteworthy feature of the cytochemical reaction was the variability of the activity pattern which was similar for each enzyme studied. The following three factors contributed to the observed variability of the staining reaction, (a). differences in the over-all activity of individual muscle cells, (b). presence of an activity gradient within muscle cells, and (c). relative prominence of mitochondrial vs. myofibrillar band activity. It should be emphasized that although the activity of certain enzymes (notably DPN diapho-

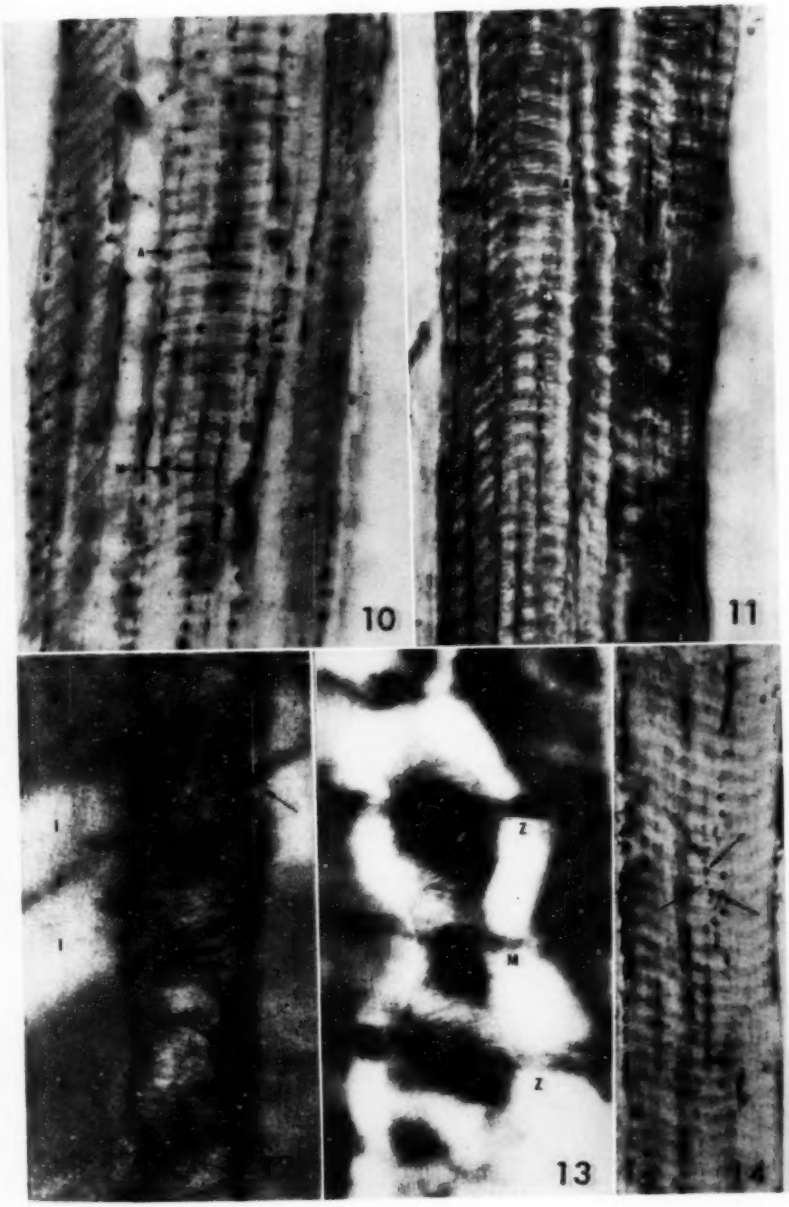
FIG. 10. Normal, unextracted sartorius stained for β-hydroxybutyric dehydrogenase. A reaction of moderate intensity was elicited from the mitochondria and a weak reaction from the A bands. Apparently, skeletal muscle may participate in the oxidative metabolism of fat. Nitro-BT method. 60 min. incubation. × 1500.

FIG. 11. Normal, unextracted sartorius stained for cytochrome oxidase. The intensity of the reaction approximates that for β-hydroxybutyric dehydrogenase (Fig. 10) or that for succinic dehydrogenase. None of these reactions were ever as intense as the reaction representing DPN diaphorase activity. Modified Nadi method. 60 min. incubation. × 1500.

FIG. 12. An electron micrograph showing portions of a mitochondrion and two myofibrils of a normal muscle. Continuity between the Z disc of the fibril on the left and the outer membranes of the mitochondria is demonstrated. The occurrence of points of continuity between the transverse system, mitochondria and the Z and M bands is consistent with their enzymatic constitution. × 70,000.

FIG. 13. An electron micrograph showing a mitochondrial fragment "suspended" in an interfibrillar space by three consecutive membranes of the transverse system linking respectively Z, M and Z bands. Apparently the mitochondrial membranes associated with the transverse myofibrillar bridges resist glycerol extraction. × 33,000.

FIG. 14. Glycerol extracted muscle stained for DPN diaphorase. The only persisting vestiges of mitochondria are represented by rows of fine dark granules, each of which is located at a point of intersection of the transverse system (at the Z and M bands) with the interfibrillar space. A similar pattern is seen in Figures 10 and 11. With the exception of where the transverse linkages are in membrane continuity the mitochondria are readily extracted by the glycerol. Nitro-BT method. 30 min. incubation. × 1500.



FIGS. 10–14

rase and DPN linked malic dehydrogenase) was decidedly more intense than that of others (e.g., succinic dehydrogenase, DPN linked isocitric dehydrogenase), the sites of the activity were invariably the same. The variability enzyme activity referred to in (a) above was observed even between cells of the same muscle section. Even neighboring cells displayed extreme differences in the degree of enzymatic activity as indicated by overstaining on the one hand and faint or no staining on the other. The gradient effect was particularly evident in normal specimens that had been incubated for 10 minutes for DPN diaphorase. The gradient of activity of this enzyme was expressed as a most intense reaction along the entire length of myofibrils at the periphery of a cell, with diminishing activity towards the axial region (Fig. 7). Such gradients were not related to thickness of the section since it was as readily demonstrated in sections of one micron thickness as in those of eight microns. The third type of variation observed in the cytochemical reaction of normal skeletal muscle was determined by the relative degree to which the mitochondria and the Z and A bands participated in the reaction (Figs. 5 and 9). In general, the mitochondrial reaction was dominant after comparatively short periods of incubation (less than 30 minutes). The reactions in the Z and A bands were often not discernible until after 30 minutes of incubation and could be displayed still more strikingly after prolonged incubations of one hour or even longer. During protracted periods (overnight) of incubation, especially in the DPN diaphorase and malic dehydrogenase reaction mixtures, the mitochondria in normal muscle specimens became over-stained and appeared larger than in specimens incubated for less than 30 minutes. This was not the case with the glycerol extracted specimens. As has been described elsewhere glycerol solutions are capable of gradually extracting from skeletal muscle the tubular longitudinal

FIG. 15. Glycerol extracted sartorius muscle as revealed by the electron microscope. The transverse membranous system crosses the interfibrillar space at successive Z and M bands as consistently repetitive as the bands themselves. The true extent and constancy of this system is not fully appreciated until after the specimen has been freed of the dense "osmiophilic" sarcoplasm and the more labile membranes that make up the sarcoplasmic reticulum and mitochondria. × 7500.

FIG. 16. At the margin of the muscle cell continuity is established between the transverse system and sarcolemma. One such connection is shown in this field. From the Z disc a double membrane (TL) arises and extends outwards for a variable distance before it becomes continuous with the sarcolemma (S). The transverse linkages (TL) are a constant feature, linking each Z and M band to the sarcolemma. The existence of these connections accounts for the festooned appearance of the sarcolemma that is demonstrable even in light microscope preparations. × 150,000.

FIG. 17. One transverse interfibrillar bridge is shown connecting Z discs of adjacent myofibrils. The sarcoplasmic reticulum (R) abuts against the transverse bridge as blind vesicles (R). The sarcoplasmic reticulum, in contrast to the transverse system, lacks oxidative enzyme activity and is not harnessed to the contractile apparatus. × 150,000.

FIGS. 15–17

FIG. 18. An electron micrograph of a portion of one muscle cell from a normal unextracted specimen of frog sartorius. In the upper left corner of the field a golgi apparatus may be discerned in the sarcoplasm adjacent to a nucleus. The transverse membranous system in step-wise fashion is seen linking together successive Z discs. The parallel component of the transverse system that connects between M bands cannot be distinguished from the sarcoplasmic reticulum when the myofilaments are so closely placed as in this illustration. The density of the Z disc may be attributed to its content of glycogen and at least the following several different proteins, that of the thin filaments (presumably actin), DPN-linked dehydrogenases, flavoprotein (DPN diaphorase) and cytochrome oxidase. The anisotropic band contains at least the thick filament protein (presumably, myosin) and coenzyme A in addition to the substances found in the Z disc. × 60,000.

TABLE I

Cytochemical Localization of Glycogen and Oxidative Enzymes in Frog Striated Muscle

	Bands				Mitochondria
	I	Z	A	H	
PAS—Glycogen (Fig. 3)	−	+	+	+	
Amylase treated control (Fig. 4)	−	−	−	−	
Succinic dehydrogenase					
Normal	−	+	+	+	+
Glycerol extracted	−	+	+	+	
Inactivated	−	−	−	−	−
Cytochrome Oxidase					
Normal (Fig. 11)	−	+	+	+	+
Glycerol extracted	−	+	+	+	
Inactivated	−	−	−	−	−
DPN Linked Enzymes					
DPN diaphorase					
Normal (Fig. 5)	−	+	+	+	+
Glycerol extracted (Figs. 6 & 14)	−	+	+	+	
Inactivated control	−	−	−	−	−
Lactic dehydrogenase					
Normal	−	+	+	+	+
Glycerol extracted	−	+	+	+	
Inactivated control	−	−	−	−	−
Malic dehydrogenase					
Normal (Fig. 7)	−	+	+	+	+
Glycerol extracted (Fig. 8)	−	+	+	+	
Inactivated control	−	−	−	−	−
Isocitric dehydrogenase					
Normal (Fig. 9)	−	+	+	+	+
Glycerol extracted	−	+	+	+	
Inactivated control	−	−	−	−	−
β hydroxybutyric dehydrogenase					
Normal (Fig. 10)	−	+	+	+	+
Glycerol extracted	−	+	+	+	
Inactivated control	−	−	−	−	−
TPN Linked Enzymes					
TPN diaphorase					
Normal	−	−	−	−	−
Glycerol extracted	−	−	−	−	
Isocitric dehydrogenase					
Normal	−	−	−	−	−
Glycerol extracted	−	−	−	−	
Malic dehydrogenase					
Normal	−	−	−	−	−
Glycerol extracted	−	−	−	−	
Glucose-6-phosphate dehydrogenase					
Normal	−	−	−	−	−
Glycerol extracted	−	−	−	−	
6-phosphogluconic dehydrogenase					
Normal	−	−	−	−	−
Glycerol extracted	−	−	−	−	

Photomicrographs illustrating cytochemical reactions are indicated in parentheses.

reticulum and mitochondria (15). That the staining ability of the myofibrillar cross banding has not been altered by the extraction procedure is evident when normal and extracted specimens are compared after staining by the Bodian protargol method (Figs. 1 and 2). In the more thoroughly extracted specimens the activity of the Z and A bands represented the dominant feature of the cytochemical reaction. In fact, only vestiges of enzymatic activity remained in the interfibrillar spaces. It is interesting that this interfibrillar activity was consistently located opposite the Z and A bands, the site, in fact, of the transverse bridges. The transverse system of myofibrillar bridges has been described in detail elsewhere (15). In Figure 15, an electron micrograph, the transverse system is seen as an extensive array of parallel membranous bridges transecting the interfibrillar spaces at consecutive Z and M bands.

The variations in reaction pattern described above which were observed in normal (intact) specimens of skeletal muscle cannot be related to the distribution of particular enzymes, but perhaps can be accounted for in terms of the over-all metabolic status of individual cells and the activity cycle of each. This will be considered further in the discussion section.

DISCUSSION

The specific localization of glycogen, DPN diaphorase, DPN linked dehydrogenases, succinic dehydrogenase and cytochrome oxidase within the A band and Z disc in frog striated muscle clearly indicates that these areas of the contractile apparatus are associated with an energy source and an enzymatic system capable of generating high energy adenosine triphosphate (ATP). The localization of these enzymes, components of the glycolytic and citric acid cycles linked to the cytochrome system, suggests that the A and Z bands as well as the mitochondria are capable of degradating glycogen to carbon dioxide and water. It is an interesting fact that isocitric dehydrogenase is linked to DPN rather than to TPN in frog muscle. In addition, it appears that fatty acids may be utilized for energy within the A and Z bands since β-hydroxybutyric dehydrogenase activity was demonstrable at these sites.

That these enzymes are strongly bound to the structures of the A band and Z disc areas is indicated by the observation that even after prolonged glycerol extraction (12 days) the enzymatic activity of the muscle striations had not been diminished. The possibility that the observed results may be artifact is remote since the enzyme localizations are consistent in both normal (unextracted) and glycerol extracted muscle and absent in the heat inactivated controls. In addition, we obtained no evidence that any component of the muscle cell can adsorb diformazan particles that had been formed exogenously. Furthermore, the myofibrillar glycogen localization corresponds exactly with the site of enzyme activity.

In 1946, Dempsey, Wislocki and Singer (16) reported on the distribution of

glycogen in the striated muscle of rats and monkeys. Glycogen was stained, after fixation in a mixture of alcohol, formaldehyde and picric acid, by Bauer's method and by the ammoniacal silver method (modified Pap's method) of Mitchell and Wislocki (17). The authors concluded that glycogen occurs in muscle without relation to the cross striations, that it may be heaped up against the sides of the muscle fibers, that it is sometimes found in large aggregates, that it may be evenly dispersed throughout the substance of the muscle fiber in both the isotropic and anisotropic segments, and that it is sometimes found piled up against the Z bands. After fixation in aqueous fixatives, and after digestion of the sections in saliva, the Z discs showed a faint Bauer reaction and were conspicuously impregnated by the silver method. In addition, they reported that not all the material stained by the Bauer and silver methods is glycogen. As these authors did not include photomicrographs in their paper, it is difficult to evaluate their results but it is possible that the use of an aqueous fixative and the relatively insensitive methods produced the erratic results. In a survey of the distribution of the periodic acid-Schiff positive carbohydrates in rats, Leblond concluded that, "glycogen was represented in cardiac and skeletal muscle as a variable, irregular staining scattering of granules or as solid stained deposits near the edges of the fibers" (18). This was not a cytological study and the description of the localization indicates that artifacts influenced his results considerably. In contrast to these early observations, the remarkable uniformity of the distribution of glycogen in the A band, Z disc and interfibrillar spaces as demonstrated in the present study cannot be interpreted as an artifact. According to unpublished observations of Walker and Bergman, glycogen is also found in the A band, Z disc and interfibrillar sarcoplasm in various striated muscles of the guinea pig, rat, and monkey, as well as in frog muscle.

Dempsey, Wislocki and Singer have also studied the cross banding of striated muscle using the Bodian protargol method and reported that a double deposit of silver occurs within each sarcomere (16). Based upon the orientation provided by the polarizing microscope these authors reported that the argyrophilic zones are located in the isotropic segment on either side of the unsilvered Z discs. In addition, the entire isotropic band appears to silver, with some accentuation along the borders that abut against the anisotropic bands. In the present study, however, the Z discs and the A bands were the only argyrophilic components of the myofibrils.

Recently Barrnett and Palade, employing the high resolution of the electron microscope, demonstrated cytochemically the existence of an enzyme localized in the M band (19). It was suggested that this so-called "M band enzyme" was in fact cholinesterase. The authors concede that, according to additional theoretical considerations, enzymes other than cholinesterase may have been demonstrated. The possibilities include deacylase, thiolase or enzyme systems

in which acetyl CoA is an intermediate. The latter alternative most readily lends itself to the results and concepts developed in the present study. Ordinarily muscle satisfies its energy needs by glycolysis. The lactic acid derived from glycolysis normally is removed from the muscle and transported to the liver for glycogenesis. From the present study it is clear that components of the citric acid cycle, DPN diaphorase, and cytochrome oxidase are also found within the A band. In addition, the myofibrillar M bands are linked to the sarcolemma by the M band transverse linkages whose role (to be discussed subsequently) may serve to activate the enzymatic cycles within the A band. It is possible therefore that the citric acid cycle may be activated by M band transverse myofibrillar bridges to utilize a fraction of the lactic acid, derived from glycolysis, for the regeneration of ATP which is constantly needed in actively contracting muscle.

Harman (20) studied the oxidative capacity of isolated muscle mitochondria and myofibrils. The skeletal muscles of pigeon breasts were homogenized, submitted to differential centrifugation and separated into purified suspensions. This author reported the absence of residual integrated oxidative enzymes associated with the isolated myofibrillar fragments while a clear demonstration of oxidative enzymes was demonstrated within the mitochondrial fraction. These observations suggested the functional dependence of the myofibrils upon energy sources probably derived from mitochondria. The results obtained by Harman probably reflect the inadequacy of the techniques then available. The use of normal and glycerol extracted intact muscle cells and the employment of the sensitive methods in this study probably account for the successful demonstration of oxidative enzymes within the myofibrillar A band and Z disc.

Harman also noted the oxidative capacity of various skeletal muscles and of the myocardium does not correlate well with mitochondrial population. Harman has shown that in muscles with the relatively highest proportion of mitochondria the oxidation per unit population may actually be less than that of muscles which have the lowest mitochondrial population. This finding suggested to Harman qualitative and quantitative differences in the mitochondria when different muscles are compared. In addition, it was pointed out that although the bulk of the oxidative enzymes may be located in the mitochondria as in other tissues, the participation of other muscular constituents must contribute to the total oxidative capacity of the cell. The results of this study suggest that the myofibrils and transverse bridges also contribute to the oxidative capacity of the cell.

Mommaerts (21) has reported an inability to demonstrate under anaerobic conditions the breakdown of ATP or phosphocreatine (PC) during a single muscle twitch. These breakdown products were not demonstrable during either contraction or relaxation and led Mommaerts to the conclusion that ATP may not be indispensable for muscular contraction. This point of view is dia-

metrically opposite to that held by most investigators. Mommaerts' observations are subject to another interpretation. From an examination of Hill's (22) and Gerard's (23) experiments on the anaerobic activity of nerve a suggestion has been made which may be applicable to the observed results in muscle. In an atmosphere of pure nitrogen both the resting heat production and the ability to conduct impulses diminishes steadily from one to two hours depending upon the animal, the temperature and the rate of oxidative metabolism resulting from activity. Thereafter "true" anaerobic metabolism appears and conduction block occurs. According to Hill's calculations all the dissolved oxygen in the nerve should have been lost within a few minutes. The relatively slow decline in heat production and loss of ability to conduct impulses is believed due to the presence within the nerve of a hydrogen acceptor or perhaps the saturation back along the oxidative chain from cytochrome oxidase, through the cytochromes, flavoprotein, DPN and finally to DPN linked dehydrogenases. Mommaerts studied the chemistry of the single twitch in a slow muscle (turtle), at a low temperature (-1 to $+$ 5°C.) and after a very brief period (½ hour) of anaerobiosis. It is very possible that the results of Mommaerts can be explained by incomplete anaerobiosis, low temperature, and/or an "oxidative reserve".

The generation of ATP by the enzymatic systems in the interfibrillar mitochondria is well known (24). The importance of enzymatic systems resulting in the generation of energy rich ATP is of great interest because of the role of ATP in muscular contraction and in the maintenance of muscle elasticity (25). It is probable from the work of H. Huxley and Hanson (26, 27 and 28) that muscular contraction occurs within the A band. H. Huxley has shown that the I band (thin) myofilaments extend into the A band as far as the H band. The resultant shortening of the sarcomere is due to the incorporation of thin I band filaments into the H band areas. It is believed that myosin is found in the A band in the form of thick myofilaments while actin is apparently found in the I and A bands as far as the H zone (26, 27, 28 and 29). Since myosin possesses an ATP splitting activity (25), it is probable that it is the functional activity within the A band which requires the high energy of ATP. It has been difficult, however, to visualize how the molecules of ATP derived from mitochondria are uniformly distributed to the distant myofilaments within myofibrils. Enzymatic systems intimately associated with the myofilaments at the site of contraction (in the A band) eliminate the necessity of ATP transport. From the observations of Caspersson and Thorell (30) using ultraviolet absorption, and of Engstrom (31) employing ultraviolet absorption and microincineration, phosphonucleotide-like substances, probably ATP and possibly other organic phosphates, are localized within the I band. If ATP is present within the enzyme negative isotropic band it may be bound to the thin (actin) myofilaments which are found in both the I and A bands. When the muscle cell is ex-

cited, resulting in the interaction of actin (thin myofilaments) and myosin (thick myofilaments), ATP may be exposed to the ATPase activity of the myosin. With the breakdown of ATP energy is liberated and the thin filaments are drawn up into the H zone of the A band. ATP is probably resynthesized within the A band and bound once again to the thin actin filaments before relaxation (withdrawal of the thin filaments from the H zone) occurs. Szent-Györgyi (25) has shown that glycerol extracted muscle cells will contract only if placed in a solution containing ATP, $MgCl_2$, and KCl. This suggests that ATP is bound in the intact resting muscle cell and is not available to the contractile proteins (the myofilaments in the A band). Szent-Györgyi hypothesized that excitation effected the liberation of bound ATP and resulted in muscular contraction.

Bergman (15) has shown in electron microscopic studies of glycerol extracted frog striated muscle that the interfibrillar mitochondria and longitudinal sarcoplasmic reticulum had been removed by the extraction procedure. The sarcolemma, transverse system of membranes and the myofibrils are relatively unaffected by the glycerol solutions. It was shown that the transverse system alone links the sarcolemma to the myofibrils at the Z disc and M band areas. In this paper, Bergman suggested that the transverse system of membranes be termed transverse myofibrillar bridges. A review of the literature reveals an older terminology first suggested by Heidenhain (32) which may be useful since it is specific. In a discussion of nomenclature Jordan (33) states that Heidenhain proposed that the transverse membranous system which is in continuity with the Z discs and with the sarcolemma be termed telophragma while the transverse membranous system in continuity with the M bands and the sarcolemma be termed mesophragma. Several investigators (32, 33, 34 and 35) employing the light microscope have described the transverse system which links adjacent Z bands to each other and to the sarcolemma and links adjacent M bands to each other and to the sarcolemma. The distribution of the transverse membranes described by light microscopy is precisely that described in the electron microscope study by Bergman. There was and still is no evidence of a continuity existing between the transverse system and any longitudinally arranged component (i.e., the tubular sarcoplasmic reticulum). In addition, it is necessary to clarify the nature of the telo- and mesophragmata in view of our present knowledge. The high resolution of the electron microscope reveals that the transverse system is similar to the sarcolemma (i.e., double membrane) and that it is membranous in the interfibrillar spaces. Where the membranes contact Z and M bands its membranous nature is modified, and in the case of frog skeletal muscle this area is crossed by thin myofilaments (Z band) and thick myofilaments (M band).

In glycerol extracted frog striated muscle examined with the electron microscope the density of the cross banding is diminished as compared with normal

muscle. The glycerol treatment evidently extracted the sarcoplasm from the A band and Z disc. It appears therefore that the enzymatic system is intimately associated with the myofilaments located in the A band and Z discs. Such an enzymatic system, which is highly organized spatially, could hardly exist loosely arranged in the sarcoplasm surrounding the myofilaments. Presumably, glycogen is localized in the sarcoplasm surrounding the myofilaments and in the A band and Z discs.

The isotropic disc, by virtue of its being enzyme and glycogen negative, isolates the Z disc which is enzyme and glycogen rich. One can only speculate about the possible role of the Z disc in the activity of the muscle cell. The results of this study suggest that the interfibrillar linkages also possess enzymatic activity. In addition, electron microscope studies of normal muscle indicate that there is a morphological continuity between the transverse linkages and mitochondrial membranes in the interfibrillar spaces when mitochondria are found between adjacent Z and M bands. Even after prolonged extraction (12 days) there is some suggestion of continuity of mitochondrial membranes with the transverse connections. This was not recognized in an earlier study by Bergman (15). The transverse linkages are indistinguishable from mitochondrial membranes in glycerol extracted muscle studied by electron microscopy which is consistent with the observation that oxidative enzymatic activity is demonstrable at both sites.

It has been shown by A. F. Huxley (36) using the microelectrode technique that local contractions can only be produced by stimulation of the Z disc area. How the electrical disturbance (the propagated sarcolemmal impulse) activates the transverse system has not yet been elucidated. According to A. F. Huxley's work stimulation of the A band area does not provoke any visible contraction. The muscle cell requires the activation of two necessarily related but distinctly different functional systems. These are: 1). the contractile apparatus which is activated via the Z disc transverse linkages from the sarcolemma and 2). the oxidative phosphorylating system within the A band which is activated via the M band transverse linkages from the sarcolemma. It is possible that the activation of these two systems is accomplished by the same enzymatic mechanism which would be highly efficient and synchronized.

According to a study in progress when normal frog sartorius muscles are bathed in physiological salt solution containing only a redox dye (nitro-BT) there is no evidence of enzymatic activity after a 5 to 10 minute exposure at 27°C. If, however, the muscle is stimulated by an electrical current (12 volts/20 per second) for a minute or even less enough nitro-BT is reduced to stain the Z discs and A bands blue. The formazan deposition occurred in the absence of exogenous substrate and enzymes. This suggests that metabolic activity may be activated by an electrical current.

The extensive, longitudinally arranged, tubular sarcoplasmic reticulum

would appear to play a role in the distribution of metabolites (glycogen) and removal of wastes (lactic acid and carbon dioxide) from the myofibrils and mitochondria to the extracellular space. There is no evidence of enzymatic activity within this sarcoplasmic component. Its discontinuity, morphological variability, isolation from the myofilaments of the myofibrils, and an inconsistent topographical relationship with the sarcolemma render the sarcoplasmic reticulum unsuitable for a role in the internal activation of the contractile and intrafibrillar metabolic systems. In addition, the disposition of the sarcoplasmic reticulum cannot account for transfibrillar activation of the myofilaments nor can it be correlated with the physiological studies of A. F. Huxley.

To summarize the hypothesis presented in this paper the sequence of events involved in the activation of the contractile apparatus may be stated as follows: The striated muscle cell membrane (sarcolemma) is activated at the motor end plate, initiating the appearance of a propagated muscle action potential. As the potential passes a Z disc the transverse Z linkages are activated. It is suggested that the internal conduction may be accomplished by an electron transferring system which may include DPN linked dehydrogenases, DPN, diaphorase and the cytochrome system. As this carrier system is also associated with the Z disc the transfibrillar activation of the myofilaments would be produced by the same mechanism. A. F. Huxley has shown that contraction occurs only if the Z band area is stimulated (36). Thus it would appear that it is the Z band, from which the I band (actin) myofilaments emanate, which initiates the contraction. This results in the foreshortening of the I band area due to the incorporation of I band myofilaments into the H zone of the A band. The basic contractile unit would be, according to this interpretation, that area between two successive M bands. If excitation of the contractile apparatus is the result of the liberation of ATP as suggested by Szent-Györgyi (25) perhaps the relative potential drop at the Z disc effected by the oxidation-reduction system is sufficient to trigger ATP release and muscular contraction. The sarcolemmal action current would next activate the M band transverse linkages. Since the contractile apparatus cannot be activated by the stimulation of the A band area, it is suggested the excitation transmitted via the electron transfer system of the M band linkages results in the activation of the A band enzymatic systems (glycolytic, citric acid, flavoproteins and cytochromes). The regeneration of ATP would be effected by oxidative phosphorylation.

SUMMARY

1). Oxidative enzymes, components of the glycolytic and citric acid cycles, DPN diaphorase, and cytochrome oxidase, have been cytochemically localized within the interfibrillar mitochondria, the Z disc and the A band of frog striated muscle.

2). Glycerol extracted muscles (12 days) show no loss of enzymatic activity

within the Z disc and A band. The interfibrillar enzymatic activity is markedly reduced except in the region of the Z disc and A band which is believed to be due to the presence of fragments of mitochondria which are in continuity with the transverse myofibrillar linkages.

3). Glycogen is bound within the Z disc, A band and is also located in the interfibrillar spaces.

4). Isocitric dehydrogenase is active as a DPN-linked enzyme in frog skeletal muscle.

5). β-hydroxybutyric dehydrogenase, was also localized within the Z disc, A bands and in the interfibrillar mitochondria. The presence of this enzyme indicates that oxidative metabolism of fatty acids may occur in the myofibrils.

6). An attempt has been made to correlate the results of this light microscope study with electron microscope observations. The functional significance of the findings is discussed and a hypothesis has been formulated concerning the possible mechanism of internal activation of contraction and the metabolic activity in muscle cells in which both oxidative enzymatic systems and transverse myofibrillar bridges would be expected to play important roles.

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ABSTRACTS OF PAPERS PRESENTED BEFORE THE MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

January 12, 1959

OBSERVATIONS ON THE MINERALIZING PROPENSITY OF URINE

WILLIAM C. THOMAS, JR. AND JOHN E. HOWARD

The composition of mineralized tissues in vertebrates consists of two phases—a mineral phase and a supporting organic phase, or matrix. These two components exist not only in the skeleton, but also in the calcification developing in injured muscles, in gallstones, and in renal calculi. One approach to the study of certain aspects of biological calcification is provided by use of cartilage matrix from ricketic rats. Such matrix will mineralize *in vitro* provided adequate concentrations of calcium and phosphorus exist in an incubation medium which approximates extracellular fluid in composition. Substances either reducing the availability of calcium ions or interfering with tissue metabolism have been shown to be inhibitory to this calcification *in vitro*.

A series of observations has been made on the effect of incubating ricketic rat cartilage in urine from normal subjects and from subjects with renal calculi. Prior to incubation all urines were rendered isotonic, adjusted to pH 7.4 with dilute acid or alkali, and sterilized by passage through porcelain filters. The urines from 14 subjects free of calculi failed to mineralize the ricketic cartilage matrix despite the existing high concentrations of calcium and phosphorus. Four of these subjects had previously had renal calculi, but were free of them at the time urines were obtained. Contrariwise, the urine from 21 patients with renal calculi *did mineralize* the cartilage matrix despite the fact that the calcium and phosphorus concentrations in these urines were not greater than in those from the non-calculous subjects. The urine from one cystinuric patient receiving alkali and another calculous subject receiving salicylamid failed to mineralize the matrix.

A number of substances which might affect the mineralizing propensity of these urines were investigated. No consistent differences were found in the concentrations of urea, magnesium, organic acids, ionized calcium or easily hydrolyzable phosphates in those urines mineralizing the matrix as compared with those that did not.

Ultrafiltration did not change normal urine to one that would calcify matrix. However, the addition of large amounts of calcium, prior inoculation and incubation with proteus vulgaris, or acid hydrolysis of normal urines did alter them so that they readily mineralized the cartilage matrix. The addition of citrate in high concentration (1,000 mg. %) or metaphosphate (2 mg. %) to urine from calculous subjects prevented matrix calcification, but methionine administration to such patients did not change the calcifying propensity of their urines.

Possibly discordant results have been obtained with urines from a number of non-calculous subjects who recently moved to the southeastern United States, an area where the incidence of renal calculi is high. Initial studies on urines from these subjects showed a failure of cartilage matrix calcification whereas urines obtained after 6 months' residence in this area regularly mineralized the matrix despite no change in concentrations of the aforementioned constituents.

As yet we have been unable to detect the factor, or factors, determining the calcifying propensity of urines studied by this technique. It is premature to suggest that the observations cited are pertinent to the genesis of renal stones. However, these studies have demon-

strated a heretofore undisclosed difference in the urine from calculous subjects as contrasted to the urine from most non-calculous subjects.

DIFFUSIBLE COMPLEXES OF METAL IONS

MACKENZIE WALSER

The physiological properties of calcium are evidently properties of the calcium ion. Calcium complexed by protein or citrate is ineffective, for example, in sustaining contraction in the frog heart. Calcium (and other metal ions) are also believed to become associated with multivalent anions in solution by purely electrostatic attraction. The physiological properties of metals in this ionized but undissociated form have not been studied.

The purpurate method of Raaflaub was reinvestigated as a means of determining free calcium ions in biological fluids. It was found that neither Na, K, Mg, Cu, Fe, Zn, Cl, HCO_3 ions, nor the dye itself caused significant errors. Using a linear equation, the relationship between the absorbance and calcium ion concentration was found to be a reproducible function of pH and ionic strength. A method was also developed for free magnesium ions, utilizing the dye Eriochrome Black T, and found to fulfill the same criteria.

These methods were employed to determine the effect on free calcium and magnesium ion concentrations of the substitution of multivalent anions (SO_4, HPO_4, and $Fe(CN)_6$) for chloride. The observed changes (in plasma ultrafiltrate and in synthetic solutions) were consistent with reported dissociation constants for these six ion-pairs. Similar observations with the frog heart method have been made in this laboratory and are to be reported elsewhere.

It is concluded that the effective concentrations of calcium and magnesium in body fluids are influenced by electrostatic association with multivalent anions, and that the extent of this change is predictable from the reported dissociation constants of each ion-pair.

Two applications of this principle are discussed. Uremic plasma was observed to contain several times as much diffusible calcium complexes as normal plasma. Part of this increment probably consists of ion-pairs with retained phosphate, sulfate, and other multivalent anions. The concentration of free calcium ions in uremic plasma is therefore lower than the total plasma calcium would suggest.

In dogs, sulfate administration markedly accelerates urinary excretion of calcium, magnesium, and radiostrontium, apparently because the ion-pairs $CaSO_4$, $MgSO_4$ and $SrSO_4$ are incompletely reabsorbed. Large amounts of calcium and magnesium may be mobilized in this manner, and the removal of radiostrontium from the circulating body fluids may be greatly accelerated.

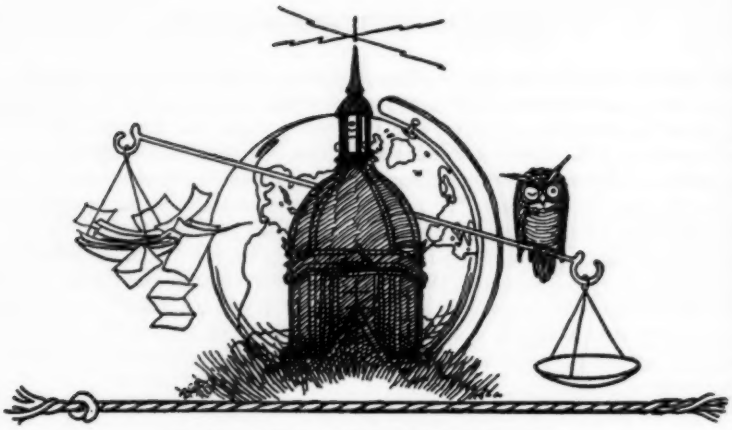
SURGICAL TREATMENT OF OCCLUSIVE DISEASE OF THE CAROTID ARTERY

FRANK C. SPENCER AND HENRY T. BAHNSON

Fifteen patients with occlusive disease of the carotid artery have been surgically treated. The occluding lesion was an arteriosclerotic plaque at the carotid bifurcation in thirteen patients; eight of these were partial occlusions and five were complete occlusions. Two patients with occlusion of the common carotid artery at its origin from the aorta due to an arteritis of unknown etiology were treated. The most common symptoms were a unilateral muscle weakness or aphasia; the symptoms were transient and recurrent in nine of the patients. Physical examination showed some neurological abnormalities in ten of the fifteen patients; examination of the carotid artery often did not show any abnormality. A systolic bruit was found in three patients. Arteriography was used to establish the diagnosis in ten patients and surgical exploration in the other five.

The patients with occlusion at the bifurcation were treated by an endarterectomy; this was successfully accomplished in the eight patients with partial occlusion. Five of these eight patients had the circulation supported with a temporary shunt while the lesion was removed. The five patients with the complete occlusion had continuity restored in only two, as in only these could back bleeding be restored; none of the patients with complete occlusion obtained any benefit from the procedure. The two patients with occlusion in the proximal carotid artery were treated by resection and insertion of an arterial prosthetic graft.

Seven of the eight patients with partial occlusion were improved from the operation; one became worse because of decreased blood flow during the operation and developed a hemiplegia that subsequently cleared after about two weeks. It was concluded from these experiences that the most favorable cases for operation were those with partial occlusion and transient symptoms but no permanent neurological injury. An endarterectomy seems to be the procedure of choice.



BOOK REVIEWS

Infrared Absorption Spectra of Steroids. Atlas Vol. 2. By GLYN ROBERTS, BEATRICE S. GALLAGHER AND R. NORMAN JONES. 95 pp., plus figs. $20.00. Interscience Publishers, New York, N. Y.

The steroids have of course become extremely important in medicine both as important constituents of the body and because of their use in therapy. Analysis of body tissues and fluids for steroid content and differentiation of each of the many known steroids calls for new and highly selective methods of analysis. Absorption spectra in the infra-red region provide remarkable possibilities for accomplishing these purposes.

However, the "fingerprint" of the spectrum, characteristic of a given steroid, must be compared with tables. Certain parts of a steroid molecule are said to have a characteristic vibrational zone in the infra-red region. Analysis of vibrational frequencies associated with these zones is complicated but provides wonderful opportunities for increase in our knowledge in this area of chemical pathology.

The present volume gives an introduction to this complicated field, then provides a table of characteristic group frequencies for steroid structure, and finally provides an atlas of 451 charts giving the curves of characteristic infra-red absorption of various compounds. The spectrum analysis is resolvable in many cases to a conclusion as to functional groups present in the molecule, a Functional Group Index of the charts is included at the back of the book. The atlas of charts is accompanied by a bibliography of 98 references.

This is one of those extremely important, highly useful, technical books needed for reference by individuals working in this field. Needless to say, it is of little use to anyone else. It constitutes a wonderful contribution from the group working under the Sloan-Kettering Institute and under the National Research Council of Canada.

F. W. BARNES, JR.

Logan Clendening Lectures on the History and Philosophy of Medicine, Eighth Series: Disease and Destiny; Logan Clendening. By RALPH H. MAJOR. 49 pp., $2.00. University of Kansas Press, Lawrence, Kansas.

In the first of the two lectures published in this booklet, Dr. Major (M.D. Johns Hopkins 1910), discusses the bearing of disease on the destiny of nations. He does this by briefly reviewing the Plague of Athens, Malaria and Roman history, the Black Death, Typhus Fever in Napoleon's Russian campaign, and the diseases of Nietzsche, Wilson, and Lenin. The second lecture deals with an altogether different subject, the life and work of Dr. Logan

Clendening in whose honor the lectureship was established. Dr. Major worked with Clendening for 25 years and the personality of his friend emerges vividly from the essay and the pictures accompanying it. Wisely, examples of Dr. Clendening's witty and forceful writings have been included. Few readers will fail to enjoy the account of a pilgrimage to Berkeley, Gloucestershire, in quest of Jenner's tomb!

OWSEI TEMKIN

Poliomyelitis. Papers and Discussions Presented at the Fourth International Poliomyelitis Conference. 684 pp., $7.50. J. B. Lippincott Co., Philadelphia, Pa.

The following "editorial note" appears on the back of the title page: "The material in this volume comprises a record of the Fourth International Poliomyelitis Conference, including a report of the opening session, the banquet and the other functions. The original presentations have been included in toto. The discussions, addresses and exhibits have been edited carefully and specially prepared for publication. All the material, delivered in several languages and transcribed by recording, has been edited in the interest of accuracy and brevity." No editorial credits are given.

This handsome volume, replete with beautifully reproduced charts and photomicrographs, was published under the auspices of the International Poliomyelitis Congress, the officers of which are all Americans and, as far as I can ascertain, high officials of the National Foundation for Infantile Paralysis (recently renamed the National Foundation). The meeting was held in Geneva, Switzerland in the summer of 1957; the exact date is not clearly stated. A portion of the proceedings are concerned with "reports of official delegates" from 35 member countries, excluding the U. S. A. but including the U.S.S.R., the colonies of the United Kingdom and the two halves of Germany considered as one, poliomyelitis-wise. These short statements afford the reader an excellent picture of the world-wide nature of the poliomyelitis problem. It is a bit unfortunate, however, that the delegates leave the impression of being cast in the role of petitioners rather than full-fledged members of the confraternity.

The scientific proceedings are another fitting testimonial to the tremendous impact on research in poliomyelitis and related fields of the triennially internationalized National Foundation. The discussions of vaccination leave little doubt of the efficacy and safety of the Salk vaccine. In addition, Koprowski and Sabin again report significant progress in development of an attentuated live vaccine for oral administration.

Perhaps the most interesting papers in this book are by-products of research in poliomyelitis. Schramm, Delbrück and Morgan present particularly lucid summaries of their respective studies to mid-1957 on chemistry, genetics and electron microscopy of viruses. Most research in these rapidly expanding fields of virology is out of date before publication, and the papers in this volume are no exception. This is also true of the reports on new enteric viruses now implicated as causes of human disease. Nevertheless, the papers are very informative and the attention of the interested readers should be especially directed to the excellent review by Dalldorf of the current status of Coxsackie viruses. Other sections of the book deal with diagnosis of viral diseases, principles of tissue culture, and the care of poliomyelitis patients with particular emphasis on respiratory complications and rehabilitation. There is ample proof here that a multifocal attack on a specific disease results in contributions to many fields of medical and biological research.

ROBERT R. WAGNER

Polysaccharides in Biology. Transactions of the Third Conference, May, 1957. Edited by GEORG F. SPRINGER. 249 pp., $4.75. The Josiah Macy, Jr. Foundation, New York, N. Y.

This volume reports the discussions held on May 29, 30, and 31, 1957, in Princeton, N. J. The two major areas covered were Homopolysaccharides (Discussion led by Dewitt Stetten,

Jr. and Marjorie R. Stetten) and Nucleotides and Saccharide Synthesis (discussion led by Luis F. Leloir). The first session of this third conference is overly long (153 pp.) and deals in too much detail with certain fine points which are likely to interest only the specialists in the field. The second session is more concise (79 pp.), much broader in scope, and likely to be of more interest to the non-specialist. Each session contains valuable semi-formal presentations by other participants, but these are very difficult to locate in the present format. The readability could be improved by setting off each semi-formal presentation with a title and including it in the Table of Contents, so that the reader could tell more readily exactly what subjects were being covered in the conference. It is definitely misleading to have only the two lead-off papers listed in the Table of Contents and set off in the text.

In the first session, data were presented by Stetten and Stetten to illustrate the heterogeneous labelling of liver and muscle glycogen by administered C^{14} glucose. The extent of labelling was reported to vary with the molecular size and with the region in a particular molecule. There was considerable disagreement between the Stettens and the Coris concerning appropriate methods for preparation of glycogen (trichloroacetic acid versus alkaline extraction) and for determination of molecular weight (light scattering vs. sedimentation-diffusion). In this connection, Roy L. Whistler discussed the chemistry of alkaline degradation of polysaccharides to isosaccharinic acid derivatives. Joseph Larner presented a review of the enzymatic techniques for structural analysis of glycogen by successive application of phosphorylase and amylo-1,6-glucosidase. He then examined the difficult question of the number of "catalytic acts per encounter" during phosphorylase action on the outer chains of glycogen, and concluded that a given encounter does not result in multiple action on a single chain. Gerty Cori reviewed briefly her elegant studies on the glycogen storage diseases, emphasizing that the term, "Von Gierke's disease", should not be applied to all of these cases, since at least four different disease entities can now be distinguished based on (a) the tissues involved (b) the structure of the glycogen and (c) the nature of the enzymatic defect. Carl F. Cori presented electron micrographs of glycogen molecules. Allene R. Jeanes reviewed the chemistry of the dextrans. Of particular interest was the demonstration of $1\rightarrow3$ linkages in addition to the known $1\rightarrow4$ and $1\rightarrow6$ linkages, and the evidence that the $1\rightarrow3$ linkage may also be present in glycogen and amylopectin. The immunochemistry of the dextrans and levans was discussed briefly by Elvin A. Kabat and Michael Heidelberger.

In the second session, Luis Leloir reviewed with characteristic modesty his important work on the role of uridine diphosphate derivatives in glycoside synthesis. In particular, he discussed the synthesis of sucrose, lactose, and glucuronides, and pointed out the probable role of the uridine nucleotides in the synthesis of simple and complex polysaccharides. Unfortunately, his new exciting studies on glycogen synthesis from uridinediphosphoglucose (UDPG) are too recent for inclusion in this conference. Herman Kalckar discussed the mechanisms of the enzymatic epimerization of UDPG to the galactose derivative, and of the enzymatic oxidation of UDPG to the glucuronide. Harland Wood described heroic isotope experiments on lactose synthesis in the cow from labelled fatty acids. The results suggest that lactose is formed from UDPG plus free glucose, rather than from UDPG plus glucose-1-phosphate as previously proposed. Saul Roseman discussed enzymes concerned in the synthesis and breakdown of acetylhexosamines. The second session closed with an interesting theory by Ernest C. Pollard concerning the factors which put an upper physical limit on the size of a glycogen molecule and which may result in its cleavage when this size is reached.

Other participants in the discussions included Paul György, William E. Ehrich, Karl Meyer, A. G. Norman, Murray J. Shear, Richard J. Winzler, Friedrich Zilliken, Michael Doudoroff, Martin B. Mathews and Frank Fremont-Smith.

The transcriptions of the discussions are remarkably lucid and free from error. This re-

viewer was particularly impressed by the accuracy with which the transcribed discussions serve to provide character sketches of the participants. It was especially interesting to read the characteristically spirited remarks of Gerty Cori, participating in what must have been one of her last scientific conferences.

SIDNEY COLOWICK

Tuberculosis, Prevention and Control. By H. W. HETHERINGTON AND FANNIE W. ESHLEMAN. 404 pp., $6.50. G. P. Putnam's Sons, including the publications of Minton, Balch & Co., New York, N. Y.

According to the map on page 10 of this text-book, Maryland remains among the six states with the highest incidence of new cases of tuberculosis and is also one of a larger group of 20 states with an annual death rate of 10 or more per 100,000 population. There is clearly, therefore, a local need for improvement in prevention and control. This text-book represents in its authorship the close collaboration between doctors and nurses which is essential for a successful tuberculosis program. Originally entitled "Nursing in Prevention and Control of Tuberculosis" it is designed mainly for public health nurses, but the fact that it has already passed through 3 editions suggests that it has reached, as it deserves, an ever wider audience. The book starts with a brief historical review of tuberculosis as an ancient and killing disease and after giving the current statistics for the United States, proceeds to a description of the symptomatology. One might quibble with a few statements, including those on fever and the statement repeated more than once that the nurse should remember that "pulmonary hemorrhage is usually less serious than it appears to be", but on the whole the statements are graphic and unexceptionable. The discussion of treatment is clear and interesting and the different drug regimes in use are outlined. The value of institutional therapy is somewhat overstressed: many of us feel that better long term results would be obtained if home and clinic treatment were more vigorously pursued at an early stage in the disease. Some of the most resistant and uncooperative patients are those whose period in an institution has been an unhappy one. The authors discuss some of the means employed in making the hospital stay more attractive but the statement that "a self discharge rate of over 50 per cent warrants investigation" admits the difficulties involved. As the spring sunshine filters in the windows the derelict tuberculous patient, so well described in this book, tends to filter out of doors, to return next winter like a dormouse or a homing swallow to the warmth and society of the City ward. Compulsory confinement of the open case is discussed without mentioning that it is not, perhaps fortunately, permissible in all states.

The nurses' role in assembling information for conferences and for follow up is excellently outlined and the meticulous description of tuberculin testing and other nursing procedures could not be improved even though the second dose of 1.0 mgm. tuberculin is considered high and is replaced in most centres by 0.1 mgm. The questions at the end of each chapter are useful for teaching. When so excellent and useful a book appears inevitably as a fifth edition, one would welcome a little more discussion of non-pulmonary tuberculosis and the epidemiology of the disease in other parts of the world: this is particularly relevant now that air travel has made tuberculosis a swift if unwelcome import: and perhaps also a more detailed outline of the speed and possibilities of successful antimicrobial therapy.

L. M. HAROUTUNIAN

Cardiovascular Sound in Health and Disease. By VICTOR MCKUSICK. 570 pp., $15.00. The Williams & Wilkins Co., Baltimore, Md.

The author of this book has given us a work of major proportions. Not only has he produced a superlative encyclopedia which illuminates every conceivable phase of cardiac and

pulmonary sound, he has also accomplished several other purposes most convincingly. In illustrating all aspects of heart disease by means of spectral phonocardiograms of excellent quality, he has boldly succeeded in gaining the prestige of his most scholarly text for a new technique which he himself pioneered. This volume places heart sound spectrography on the map in a most authoritative manner. We are left with no doubt as to the versatility of this method in depicting acoustic phenomena.

The volume has been conceived on a grand scale. It moves from a detailed, beautifully illustrated history of cardiovascular sound to basic physiologic principles. From there it moves on to the physiology of hearing, to the art of auscultation, and to phonocardiography itself. Nowhere does the author miss an opportunity to indulge his love of meticulous scholarship, of historical lore, and above all, physiology. He is never content with mere empiricism. Where anatomy can add to understanding, the illustrations are profuse and clear. Portraits, diagrams, charts, drawings, photomicrographs, tables and detailed captions for all figures are presented in abundance. Numerous clinical examples are cited from the author's own clinical experience. Even a little Gallic spice in the form of a few satiric cartoons is provided.

The volume is beautifully edited and printed and has a bibliography of unmatched completeness—over 1600 titles. There is no doubt that this is and will be the authoritative treatise on heart sounds for a long time to come.

ABRAHAM GENECIN

Breast Cancer. The Second Biennial Louisiana Cancer Conference. Edited by ALBERT SEGALOFF. 257 pp., $5.00. The C. V. Mosby Co., St. Louis, Mo.

This book represents a report of a symposium on breast cancer which was organized as the Second Biennial Louisiana Cancer Conference under the auspices of the Louisiana division of the American Cancer Society.

There were a number of distinguished participants who represented many of the specialities concerned with breast cancer problems. Included were surgeons, radiologists, pathologists, physicians, endocrinologists, epidemiologists, biochemists, zoologists, urologists, and psychiatrists.

The Conference was arranged so that many facets of breast cancer were presented by persons of authority, and this was followed by a panel discussion which is recorded verbatim. The material of the talks was current, but there was nothing new or original which had not been presented elsewhere previously. The discussions are, for the most part, good, and indicate how controversial many of the matters related to breast cancer are.

The bibliography is good. The volume should be useful as a reference for those concerned with the treatment of patients in any stage of breast cancer.

WILLIAM F. RIENHOFF, III

Water and Electrolyte Metabolism in Relation to Age and Sex. Ciba Foundation Colloquia on Ageing, Vol. 4. Edited by G. E. W. WOLSTENHOLME AND CECILIA M. O'CONNOR. 327 pp., $8.50. Little, Brown & Co., Boston, Mass.

The symposia conducted by the Ciba Foundation have provided valuable exchange of ideas in a number of fields during the past decade. The meeting which forms the subject of this volume was held in January, 1958, and included two dozen participants interested in various phases of water and electrolyte metabolism. As might be anticipated, only a minority of those attending were primarily interested in the relation of age and sex to electrolyte metabolism, and the reorientation of many of the 18 papers to this topic was not uniformly successful. It is also unfortunate that only occasional attempts were made to discriminate

between the role of body size, body fat content, and varying intake, on the one hand, and immaturity or senescence on the other in determining the age differences described. No physiological explanation was suggested for most of the sex differences found. Approximately one half of the papers are therefore useful only as empirical accounts of certain parameters of water and electrolyte handling under various conditions.

Important contributions are presented by Wallace, Weil, and Taylor, who determined the effect on body composition in rats of varying intakes of protein and salts; by G. C. Kennedy, who used nucleic acid phosphorus fractions in kidney tissue as an index of hyperplasia, and by Krecek and associates from Prague, who studied the actions of adrenal and pituitary hormones in infant rats. Interesting reviews are given by Fourman and Leeson on the subject of hypernatremia and hyponatremia and by Kerpel-Fronius on the clinical significance of the differences between infants and adults in hemodynamics, fluid spaces, and renal function. Many of the most stimulating aspects of this book will be found in the discussions which follow each paper.

MACKENZIE WALSER

Callander's Surgical Anatomy, 4th edition. By BARRY J. ANSON AND WALTER G. MADDOCK. 1157 pp., $21.00. W. B. Saunders Co., Philadelphia, Pa.

Almost 40 years have elapsed since Dr. C. Latimer Callander and his father, Dr. Cyrus Newton Callander, conceived the basic idea of a text which would "present anatomic facts in terms of their clinical importance". Having now been in print for twenty-five years, this text is well established as a standard in the field and it would seem that its continuing popularity is in no small part due to the observance of the basic plan noted above. The present revision, as well as the third edition which was published in 1952, has been carried out by Drs. Anson and Maddock who have again done an excellent job of revising the text and illustrations in accordance with the newer developments in the field of surgery. The anatomical material has been supplemented by improved illustrations and the text revised as necessary. The pattern of presentation remains unchanged; anatomic considerations are organized on a regional basis and the pertinent surgical discussions directly follow the anatomic material. The clinical segments of the text are not encyclopedic but include the important basic information and rationale of therapy. The scope of consideration is complete, including the fields of the surgical specialties as well as general surgery.

The present edition should be valuable to both student and surgeon and should retain its present high place in the literature of this field.

JAMES R. CANTRELL

To Work in the Vineyard of Surgery. The Reminiscences of J. Collins Warren, 1842–1927. Edited, with Appendices, Notes and Comments by EDWARD D. CHURCHILL. 288 pp., $6.00. Harvard University Press, Cambridge, Mass.

The reminiscences of J. Collins Warren as edited by his successor—several generations removed—make delightful reading and are an important contribution to the history of the period of surgery's greatest advances, from the introduction of anesthesia through the adoption of aseptic technique and the accomplishment of healing without infection. Six generations of Warrens have practiced medicine in Boston, and except for the first who was killed on Bunker Hill all have been teachers at Harvard. J. Collins Warren (1842–1927) left reminiscences of his times and association with medicine, Boston and Harvard, wrote of his early training, his trips to Europe the then center of surgical education, and later his introduction of asepsis to Boston and work with reorganization of Harvard Medical School. The rich annotations by Dr. Churchill, present Homans Professor at Harvard and Chief of Surgery at

Massachusetts General Hospital, add immeasurably to the value and pleasure of the chronicle.

Readers with a liking for history, Harvard, Boston, or the Massachusetts General Hospital will enjoy this short book.

HENRY T. BAHNSON

Diseases of the Esophagus. By J. TERRACOL AND RICHARD H. SWEET. 682. pp., $20.00. W. B. Saunders Co., Philadelphia, Pa.

The wealth of material presented in Professor J. Terracol's LES MALADIES DE L'OESOPHAGE has been made readily available by Dr. Richard H. Sweet's translation. This is not merely a translation but is a revised and condensed edition.

The book opens with chapters on the anatomy and physiology of the esophagus followed by discussion of the diagnostic use of radiology, esophagoscopy and bongienage.

The second section of the book deals with clinical and therapeutic considerations of esophageal disease. The subjects covered range from congenital anomalies and motor disturbance on one hand to syphilis, extraction of foreign bodies and malignant tumors on the other.

The emphasis in this book is more anatomic than physiologic. In fact five times as many pages are devoted to the anatomy of the esophagus as to its physiology. This slant is also illustrated in the chapter on Mega-Esophagus (Achalasia) in which Dr. Sweet has included a lengthy discussion of his division of achalasia into two clinical types, yet devotes only a scant paragraph to the recent studies of esophageal motor function in this disease

This valuable book contains much information on the esophagus not readily available in English and a seventy-five page bibliography.

THOMAS R. HENDRIX

BOOKS RECEIVED FOR REVIEW

The Biology of Hair Growth. Edited by WILLIAM MONTAGNA AND RICHARD A. ELLIS. 520 pp., $15.00. Academic Press, New York, N. Y.

Bone Tumors, 2nd edition. By LOUIS LICHTENSTEIN. 402 pp., $12.00. The C. V. Mosby Co., St. Louis, Mo.

Ciba Foundation Symposium on Amino Acids and Peptides with Antimetabolic Activity. Edited by G. E. W. WOLSTENHOLME AND CECILIA M. O'CONNOR. 286 pp., $8.75. Little, Brown & Co., Boston, Mass.

Clinical Orthopaedics No. 12. Rehabilitation. Edited by ANTHONY F. DE PALMA. 327 pp., $7.50 single copy. J. B. Lippincott Co., Philadelphia, Pa.

Convulsive Disorders of Children. By DORA HSI-CHIH CHAO, RALPH DRUCKMAN AND PETER KELLAWAY. 151 pp., $6.00. W. B. Saunders Co., Philadelphia, Pa.

Fracture Surgery. A Textbook of Common Fractures. By HENRY MILCH AND ROBERT AUSTIN MILCH. 470 pp., $17.50. Paul B. Hoeber, Inc., New York, N. Y.

Long-Term Illness. Management of the Chronically Ill Patient. Edited by MICHAEL G. WOHL. With the Collaboration of Seventy-Nine Contributing Authorities. 748 pp., $17.00. W. B. Saunders Co., Philadelphia, Pa.

The Middle Ear. By HEINRICH G. KOBRAK with a Foreword by JOHN R. LINDSAY. 254 pp., $15.00. The University of Chicago Press, Chicago, Ill.

Practical Dermatology, 2nd edition. By GEORGE M. LEWIS. 363 pp., $8.00. W. B. Saunders Co., Philadelphia, Pa.

Surgery in World War II. Vol. I. Neurosurgery. Prepared and published under the direction of Maj. Gen. S. B. HAYS, The Surgeon General, U. S. Army. Editor in Chief, COL. JOHN BOYD COATES, JR. Editors for Neurosurgery, R. GLEN SPURLING AND BARNES WOODHALL. Associate Editor ELIZABETH M. MCFETRIDGE. 466 pp., $5.00. Superintendent of Documents, Government Printing Office, Washington, D. C.

A Symposium on the Chemical Basis of Development. Sponsored by The McCollum-Pratt Institute of The Johns Hopkins University with support from The National Science Foundation. Edited by WILLIAM D. MCELROY AND BENTLEY GLASS. 934 pp., $15.00. The Johns Hopkins Press, Baltimore, Md.

Vascular Surgery. By GEZA DE TAKATS. 726 pp., $17.50. W. B. Saunders Co., Philadelphia, Pa.

Viral and Rickettsial Infections of Man, 3rd edition. Edited by THOMAS M. RIVERS AND FRANK L. HORSFALL, JR. 967 pp., $8.50. J. B. Lippincott Co., Philadelphia, Pa.

MICROELECTRODE STUDIES OF THE COCHLEAR NUCLEI OF THE CAT[1]

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INTRODUCTION

The aim of this paper is to present some findings pertaining to single neurons in the cochlear nuclear complex of the cat. Since some properties of these cells are already known (6, 8, 12, 25), we shall be concerned with problems not investigated previously and with data where our findings enlarge or modify the existing knowledge on the subject.

We shall concern ourselves first with the matter of tonotopical organization. On the basis of cytoarchitectonic considerations the cochlear complex will be divided into three major nuclei, and we shall show that a tonotopical organization is present in each of them. We shall deal next with the response areas of single elements to tones. It will be shown that units with narrow or wide response areas coexist in the cochlear nuclei, and we shall present some quantitative data on this matter. We shall also describe the adaptation behavior of the units. Evidence will be presented to show that intensity of the stimulus is one of the important variables which determine the course of adaptation, and a simple interpretation to account for the observed differences in the adaptive behavior of various single elements will be proposed. We shall further consider some suppression phenomena not hitherto described. It will be shown, for a certain type of units, that the best excitatory frequency may become, for a time, an inhibitory stimulus, when the tone is intense. We shall finally deal briefly with the responses of the units to clicks.

Some of these findings have been already reported in preliminary communications (10, 22).

It is a pleasure to acknowledge here the technical assistance of Mr. Allen Rupert in both the experimental procedures and the analysis of the data.

MATERIAL AND METHODS

Thirty-four cats were used in two experimental series consisting of 13 and 21 animals respectively. These series will be referred to as the 1st and 2nd series.[3] The animals were deeply anesthetized with dialurethane (1st series) or

[1] This investigation was supported in part by research grant B-357 from the National Institutes of Health, Department of Health, Education and Welfare.

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[3] The first series of experiments was performed in the Psycho-Acoustic Laboratory, Harvard University, Cambridge, Mass.

sodium pentobarbital (2nd series). The external meatus was dissected free and cut transversely. For the 1st series a 6 cm. length of plastic tubing (with 3 cm. length of cotton yarn to attenuate the resonant peaks) was inserted into the canal. In the 2nd series the animals were mounted in a modified Horsley-Clarke instrument and the hollow ear bars were utilized as the sound path. A pair of Permoflux (PDR-10) earphones mounted firmly near the head of the animal and connected to the plastic tubing or the hollow ear bars presented the stimuli. Both tympanic bullae were opened in all experiments and an electrode placed at the round window. After the appropriate portion of the skull was opened part of the cerebellum overlaying the cochlear nuclei was removed. A segment of the dorsal cochlear nucleus was usually exposed to permit placing the electrode under visual control with the use of Zeiss operating microscope.

The recording and stimulating equipment for the 1st series was the same as used in the previous work (7, 9, 21). For the 2nd series stimuli were generated by the Grason-Stadler devices transduced by PDR-10 earphones. Tonal output of this system is flat with $\mp$ 5 db. over the frequency range of 100 to 5000 cps at the standard 0.1 v. reference level. Above 5000 cps the sound output drops off at a rate of about 20 db. per octave. Clicks were generated usually by 0.075 msec. square waves. Some characteristics of the click stimuli generated by the devices used have been reported elsewhere (19). Both click and tone (at 2.0 kc.) had approximately the same thresholds for a normal human ear.

Tasaki cathode-follower and Tektronix 122 amplifier were used for recording. The signals were displayed on a 4-channel oscilloscope (Electronic Tube Corporation) and the traces photographed with a Grass (C 4A) camera.

In the first series the electrode used was a glass micropipette filled with normal saline. In the 2nd series an indium filled glass micropipette was employed with a gold and platinum-black tip of 3–6 μ in diameter (3). All records shown were obtained using this electrode.

All experiments were done with animals in a sound isolated room. During the 1st series of experiments it was necessary to enter the room to advance the microdrive carrying the electrode. For the 2nd series the electrode was advanced by a system of oil-filled syringes the operation of which was controlled from the recording room.

The thresholds for clicks at the round window (first neural response) was determined before the beginning of the experiment and at frequent intervals thereafter. Experiments were carried out only on animals with low threshold for neural click response. If this threshold rose markedly during experiment (as happened occasionally), the experiment was terminated. With the sound system used in the 2nd series the threshold for clicks was usually around 100 db.

After conclusion of an experiment the animal was perfused with normal

saline and 10 per cent formalin. The brainstem containing the cochlear nuclei was embedded in paraffin, sectioned serially at 30 μ, and stained with thionin. Every section was mounted. Since it was our custom to advance the electrode throughout the entire responding tissue the identification of the electrode track and determination of its actual length present usually no major problems. Difficulty in identification of the track or a given locus along it arises if the track is shorter than about 2 mm., if the electrode enters the cochlear nuclei through overlying structures and finally if the electrode creases rather than enters the complex and slides along its edge.

Determination of response areas. All determinations of response areas were done using short tone pips. The procedure employed was to determine first the best frequency of a unit, i.e., this frequency which activated it at the lowest intensity. The effective tonal bands at successively higher intensity levels (usually in steps of 10 db.) were then determined. All determinations of this type were done by listening to the discharges. Unless otherwise stated the intensity of the sound is expressed as attenuation in db *below* our standard (Odb) reference level.

Certain features are common to all illustrations. All findings pertain to discharges in the cochlear nuclei homolateral to the stimulated ear. The negative deflection is up in all records. The experimental animals in the 1st series are identified by a letter P, those in the 2nd series by letters CN. The arabic numerals following the letters identify the animal; subscript identifies a unit in a given experiment.

RESULTS

I. Anatomical Considerations

1. Macroscopic appearance of the cochlear nuclear complex

The cochlear nuclear complex of the cat forms upon the brainstem a protuberance which is readily seen macroscopically after removal of the cerebellum (Fig. 1). The complex somewhat resembles a boomerang in shape since it possesses an anterior or horizontal arm (the long axis of which is roughly parallel to the long axis of the brainstem) and a posterior arm which ascends steeply on the restiform body from lateroventrad mediodorsally. It is customary to distinguish within the complex the dorsal cochlear nucleus (acoustic tubercle) which forms the ascending arm of the macroscopically visible protrusion and a ventral ganglion. The latter forms the anterior arm of the protuberance but reaches posteriorly medial to the dorsal cochlear nucleus and is thus partly overlaid by it.

2. Cytoarchitecture of the cochlear nucleus complex

The dorsal cochlear nucleus is usually treated as a morphological unit. The ventral ganglion has been often subdivided into two or three segments. How-

FIG. 1. Lateral view of the right cochlear nuclear complex of the cat after removal of the homolateral cerebellum. The restiform body and the superior cerebellar peduncle have been cut at a. b = cut edge of the brachium pontis. The eighth nerve (8th n.) has been cut in two steps. Behind the fifth nerve (5th n.) and ventral to the cochlear complex the trapezoid body is seen. Immediately behind the cochlear complex the free surface of the brainstem consists of fiber tracts forming the restiform body. The dorsal extension of the cochlear complex is formed exclusively by the dorsal cochlear nucleus. The latter forms also the posterior portion of the complex further ventrally but overlies here the posteroventral nucleus (see text). The anterior portion of the complex is formed by the anteroventral nucleus.

ever, it is possible to make finer subdivisions and on the basis of his Golgi material, Lorente de Nó (17) recognizes thirteen separate segments. Although Nissl preparations do not at all reveal the complex morphological make-up of the system, they seem to provide a clue for anatomical determination of the functionally meaningful major subdivisions. Cytoarchitecturally, we distinguish three configurations. Their recognition is both essential and sufficient, we believe, for the interpretation of our experimental findings. All three can be seen in one section in appropriate sagittal preparations. They are: the dorsal cochlear nucleus (Dc), the posteroventral nucleus (Pv) and the anteroventral

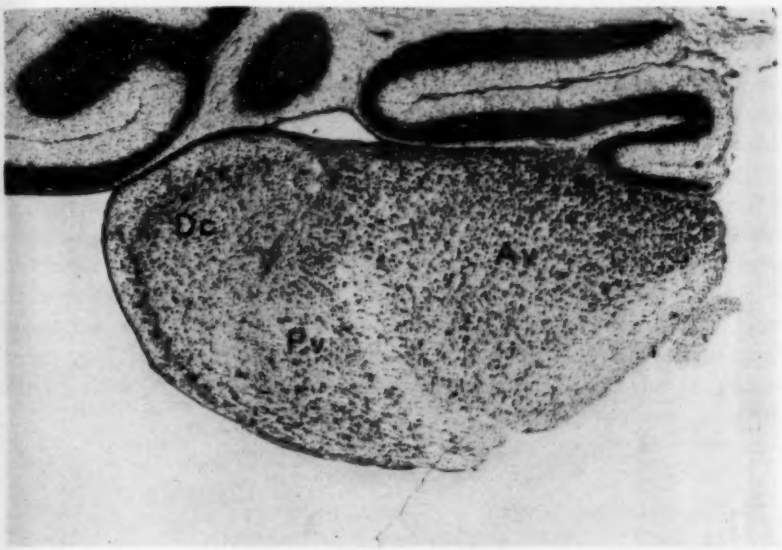

FIG 2. A sagittal section through the right cochlear complex of the cat showing the anteroventral nucleus (Av), the posteroventral nucleus (Pv) and the dorsal cochlear nucleus (Dc). Note the typical triangular shape of the posteroventral nucleus in this plane of sectioning and the fibrous lamella separating Av from Pv. Note also the Av is not uniform cytoarchitecturally. Thionin stain, × 22.

nucleus (Av) (Fig. 2). No detailed description of cells seen in Nissl preparations needs to be given here since such description has been presented by Fuse (5). However, some characteristics must be mentioned in order to make clear the proposed parcellations.

a. The dorsal cochlear nucleus (*Dc*). This nucleus is distinctly laminated. Apart from the ependymal cell layer which covers its free surface, a molecular layer, a pyramidal layer and a polymorph layer are usually distinguished. The molecular layer consists of a fair number of small granular cells and a small number of larger cells. The pyramidal layer lends the structure its characteristic appearance since it is formed by a garland of radially oriented large cells among which a large number of small granules is interspersed. The polymorph layer consists of loosely scattered, large and medium-sized cells.

b. The posteroventral nucleus (*Pv*). Ventro-orally to Dc, there extends an area of large, loosely scattered cells which we designate as posteroventral nucleus. Anteriorly the nucleus extends to the fibrous region formed by the entering and bifurcating cochlear nerve fibers. Pv has a pyramidal shape pointing with its sharp end dorsally towards the region of junction of Av and Dc.

c. The anteroventral nucleus (Av). Av of our designation lies oral to the dorsal cochlear and posteroventral nuclei. It is apparent from Figure 2 that the cell composition of the Av is not uniform and that its cells become smaller and are more densely packed as one approaches the oral pole of the region. It is, nevertheless, expedient to consider at present this whole region as a unit.

Figure 2 indicates a heavy accumulation of small granular cells at the border of Dc and Av. Small granules are seen separating also, at least in part, Dc from Pv. Such a heavy accumulation of granules in these regions is typical and a similar heavy accumulation of small granular cells forms a continuous layer along the lateral free border of the anteroventral and posteroventral nuclei. (Der laterale Körnerdamm of Fuse). Dc does not display such a layer of granular cells at its free edge but the molecular and pyramidal layers both contain, as mentioned, numerous small granules. Moreover, such granules form occasionally conspicuous nests in its molecular layer.

II. Experimental Results

1. The tonotopical organization of single elements

All microelectrodes penetrated the cochlear nuclear complex in some variant of a dorsoventral or horizontal direction. If from above, the electrode traversed either Dc alone or this nucleus and Pv in succession. If from behind, the electrode penetrated successively Dc, Pv and finally Av. Almost all insertions were done through Dc since only this nucleus was usually visualized during the experiments.

a. The existence of tonotopical organization in the individual cochlear nuclei. A typical sequence of best frequencies is seen in Figure 3. It is apparent that an orderly progression of best frequencies exists, that the best frequencies drop from high to low, and finally that *one* orderly sequence is present in Dc and *another* in Av. The electrode actually passed, in this experiment, through the uppermost tip of Pv but no units were isolated during the passage through this region. Figure 4 illustrates data from another experiment. The electrode penetrated Dc somewhat more laterally and ventrally to the penetration shown in Figure 3. The first best frequency near the surface is 12.0 kc. There is again a progressive drop in best frequencies throughout the passage of the electrode through the Dc. Upon entry into the dorsal tip of Pv an impressive jump in best frequency to about 60.0 kc. occurs and all four units in this region have best frequencies not lower than 30.0 kc. With our sound system, it would be injudicious to maintain that the best frequencies of these units were necessarily as stated. Undoubtedly, however, they were very high. Upon entry into Av a slower progressive drop in best frequencies commences again and finally reaches the frequency of 4.2 kc. in the oroventral pole of the nucleus. It is clear from the data that two sequences of best frequencies certainly exist. It

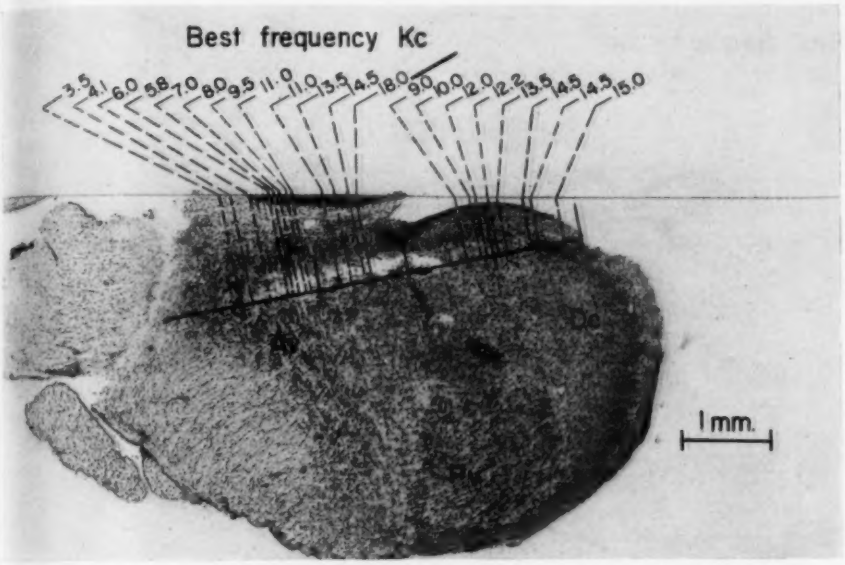

FIG. 3. Sagittal section through the left cochlear complex (the oral pole of the complex is to the left) in cat CN 16 showing part of the electrode track which is seen as a strip of destruction just above the solid black line indicating the direction and the length of the track. In this and other similar figures the section is so chosen that the destruction due to the shaft of the electrode obscures as little as possible the cytoarchitectonic picture and the electrode track is projected on a single section. The value of a millimeter along the track is shown by solid bars. Dashed lines indicate the depth at which a given neuron was encountered by the electrode and point to the best frequency determined for this neuron. Note the orderly drop in best frequencies from 15.0 to 9.0 kc. as the electrode passes the dorsal cochlear nucleus (Dc). Note further that the best frequency for the first unit in the anteroventral nucleus (Av) is 18.0 kc. and that a new orderly sequence from high to low is evident as the electrode advances.

seems probable, moreover, that a third may be present if the succession of the very high frequency units in Pv is counted as an independent sequence.

That the latter interpretation is probably correct is shown by data in Figure 5. Here the electrode enters Dc still further laterally and ventrally than is the case in the experiment illustrated by Figure 4. The initial best frequency is now 7.8 kc. Again there is a drop to about 4.0 kc. during the passage of the electrode through Dc. The electrode then enters the trigone of Pv. Four units are isolated in quick succession; and all four display best frequencies of the order of 30.0 kc. As soon as the electrode enters Av the best frequencies drop again to very much lower values. Figure 6 indicates that the best frequencies of units in Pv are by no means always high. The electrode enters Dc quite dorsally. There is the familiar sequence from high to low best frequencies in

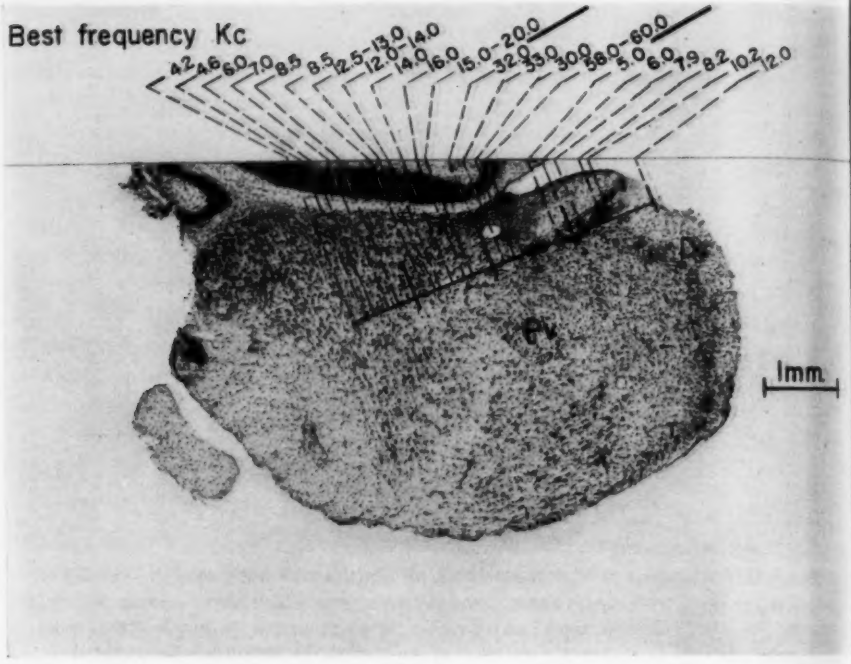

FIG. 4. Sagittal section through the left cochlear complex in cat CN 17. The electrode enters the dorsal cochlear nucleus somewhat more laterally and ventrally than in Figure 3. Note the progressive drop of best frequencies from 12.0 to 5.0 kc. in Dc, the very high best frequencies for four units in the dorsal tip of the posteroventral nucleus (Pv) and again a progressive decline in best frequencies in Av. All arrangements as in Figure 3.

Dc; a jump to higher frequencies upon entry into Pv; and a rapid drop in best frequencies as the electrode moves ventrally in the latter.

Figure 7 illustrates an almost straight dorsoventral penetration in a frontal section. The electrode enters Dc almost at its lateral and ventral edge. The best frequency at entry is 0.7 kc. Even so, there is a rapid drop in best frequencies as the electrode is advanced, the lowest value reached being 0.14 kc., which is the lowest best frequency encountered in our material.

It will be noted in Figure 7 that as soon as the electrode leaves Dc there is a jump to higher values although at least the first two best frequencies depart from the usual regular sequence. Nevertheless, when the electrode is definitely in Pv a regular sequence from high to low starts again and the lowest best frequency reached is 0.6–1.5 kc. It may be observed that the short sequence of rising rather than dropping best frequencies in Figure 7 appears to occur when the electrode is passing in the vicinity of the granular layer visible

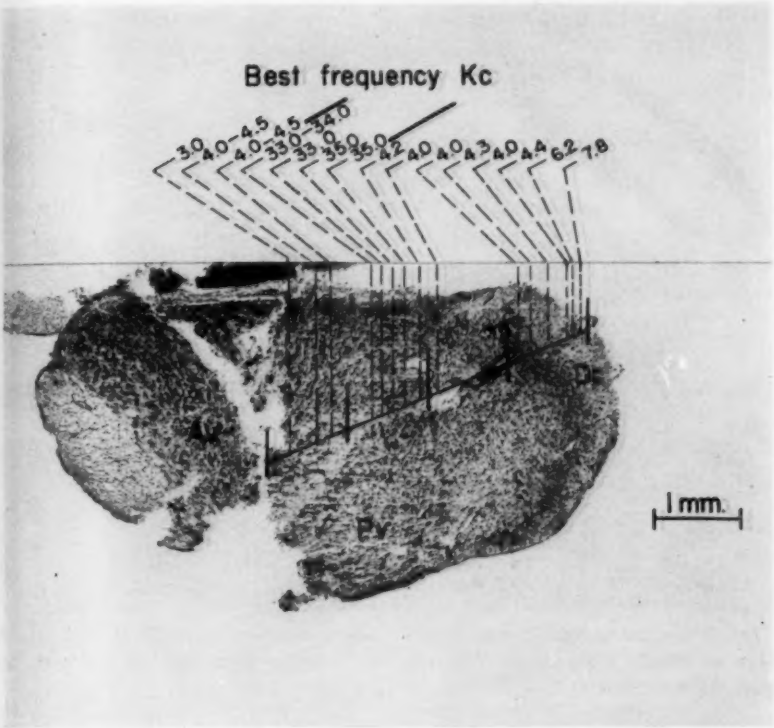

FIG. 5. Sagittal section through the left cochlear complex of cat CN 18. The electrode enters the complex still more ventrolaterally than is the case for Figure 4. Observe the progressive drop in best frequencies in Dc, the sequence of high frequency units in dorsal part of Pv, and a short sequence of low best frequencies on Av. All arangements as in Figure 3.

at the lateral edge of Pv. A similar short sequence of rising best frequencies was observed on one other occasion when the electrode was passing the region of the small granules. Whether this is coincidental or whether the region of the small granules displays its own best frequency sequence must be left unanswered at present.

The data just presented are typical for our material which consists of over 550 best frequencies determined during 32 penetrations in 27 animals. The units in each of the three divisions of the cochlear complex are almost invariably arranged in an orderly fashion as far as their best frequencies are concerned and the anatomical limits of each division are remarkably well correlated with each regular sequence. The occasional departures from such a sequence are usually so small that we feel justified in neglecting them altogether. We believe it reasonable to conclude, therefore, that a precise

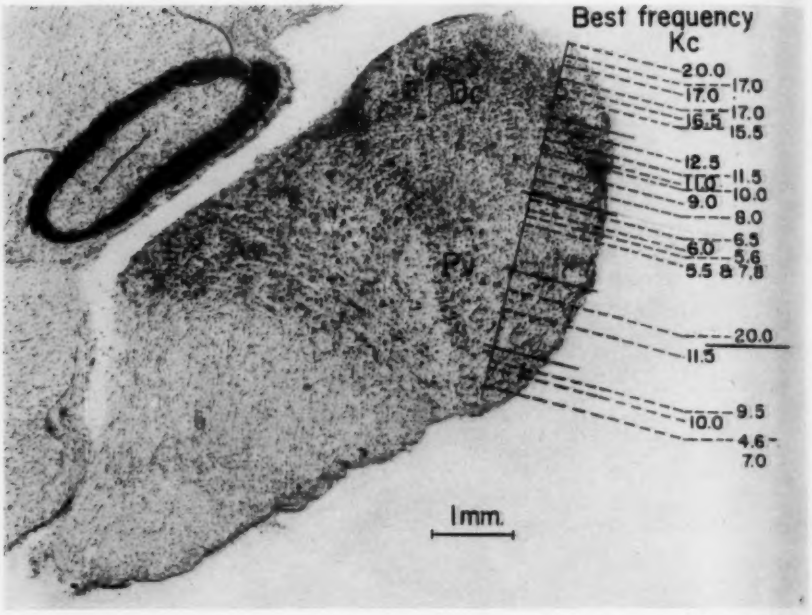

FIG. 6. Sagittal section through the left cochlear complex in cat CN 8. The electrode enters Dc and Pv in succession. Notice the two sequences from high to low in both these nuclei. All arrangements as in Figure 3.

tonotopical organization exists in each major division of the cochlear nuclear complex.

b. The detailed arrangement of the frequency sensitive units. While the existence of tonotopical organization seems well established by our data the precise topographical arrangement of tone sensitive elements is less clear. Nevertheless, certain general principles in regard to this organization emerge.

α. The anteroventral nucleus. It is uncertain from consideration of Figures 3 and 4 whether the progressive lowering of best frequencies is related to the advancement in the oral or in the ventral direction. Figure 3 alone could lead one to presume that the caudo-oral penetration is crucial. However, when all the relevant data are considered a consistent fit is obtained on the assumption that the frequency sensitive units are arranged in principle from high to low in the dorso-ventral sequence. This assumption agrees well also with one experiment (Cn19) in which the electrode did not properly enter Av but was sliding along its dorsal edge. While the response areas for units were remote and indistinct all the best frequencies were high (25.0–40.0 kc.) as would be expected if the high frequency elements are situated dorsally. The material at hand is insufficient for any detailed considerations in regard to the relative volume devoted to representation of the different frequencies.

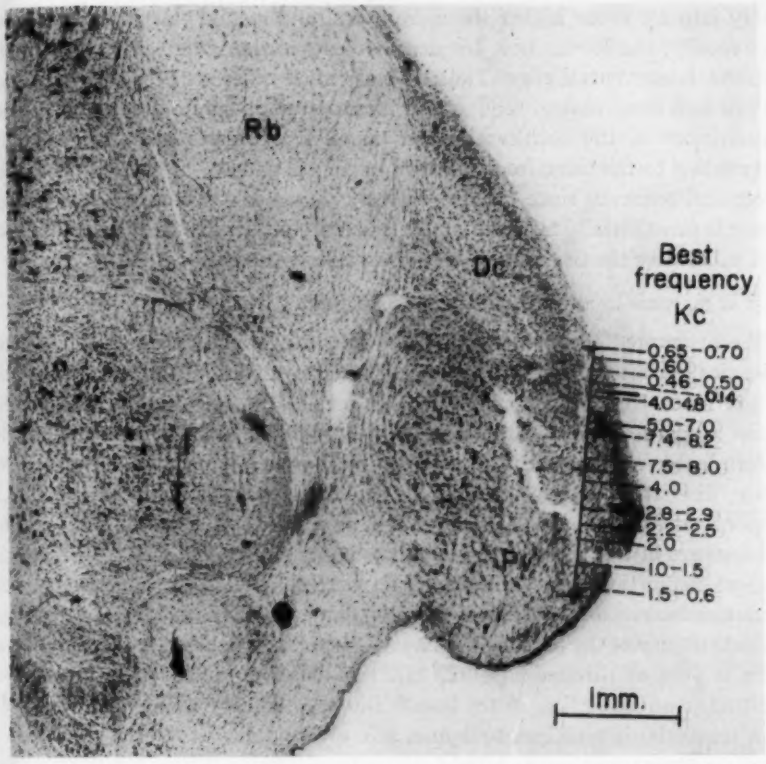

FIG. 7. Frontal section near the posterior end of the cochlear complex in cat P 95. The electrode enters the complex at the lateroventral edge of the dorsal cochlear nucleus. Note the regular drop in best frequencies as the electrode traverses Dc and the low values of these frequencies. Note also the irregular sequence which occurs immediately ventral to the border of Dc (marked by a bar) and a regular sequence of dropping best frequencies as the electrode progresses through Pv. Rb = restiform body. All arrangements as in Figure 3.

β. The posteroventral nucleus. It is apparent from Figures 4 and 5 that the dorsal tip of Pv contains exclusively high frequency elements. As is the case for Av all data are consistent with the assumption that high frequencies are represented dorsally and low frequencies ventrally.

γ. The dorsal cochlear nucleus. Since, as has been mentioned, almost all electrodes entered the cochlear complex through Dc our information concerning tonotopical organization of the latter is relatively abundant. It will be recalled that Dc is a thin, elongated structure which extends steeply on the restiform body from lateroventrad–mediodorsally. Hence an electrode entering its main segment in a dorsoventral direction is likely to slide more or less along its pyramidal layer. There is little doubt that the best frequency at the point of

entry into Dc is the higher the more dorsal and medial this entry is located. Conversely, the lowest best frequencies were encountered upon entry at the extreme lateroventral edge. Thus the individual units are arranged from high to low in a dorsoventral sequence in harmony with the findings for the other subdivisions of the cochlear nuclear complex. It is probable that the cells responding to the same frequency are arranged, at least approximately, in an orocaudal sequence since the drop in octaves per one millimeter of traversed tissue is substantially faster when the electrode penetrates dorsoventrally than if it penetrates the tissue in a plane approaching the horizontal.

2. The response areas of frequency sensitive units

We attempted to determine for the units studied not only their best frequencies but also their responses to the tonal spectrum at different intensities of the tones. Technically, it is often not feasible to determine accurately the entire response area of a single element even if its best frequency is readily determinable since, unless the unit has been completely isolated by the electrode, discharges of other units may be recruited by loud tones and thus a response area of a cluster of units rather than that of a single element results. All findings in this section pertain to a sample of 75 units in regard to which we feel secure that only one element respectively was responding at all times.

a. Continuous and interrupted effective tonal bands. It is well established that if the intensity of the tone is raised a unit responds to a broader tonal spectrum than it does at threshold (8, 12, 25). For many units the determination of limiting frequencies (i.e., of the lowest and the highest frequency to which the unit responds) is sufficient to define, at a given intensity level, the frequency response since tones between the limiting frequencies will also be effective. For many units, however, this is not the case and one or more tonal bands situated between the limiting frequencies may be quite ineffective in arousing discharges. Such ineffective tonal bands tend to occur for moderately intense or intense tones and often disappear when intensity level is raised still further. It is, of course, apparent that deficiencies of the stimulating system can be responsible for ineffectiveness of certain tonal bands. Nevertheless, we think it unlikely that faulty delivery of the stimuli could altogether account for the occurrence of ineffective tonal bands since such bands vary greatly in width and location in the tone spectrum for different units even in the same animal, they occur at different intensity levels, and they need not be correlated with the deficiencies of the sound producing system. Whatever the proper interpretation may be of the ineffective tonal bands, the units which respond to all tones between the limiting frequencies and those which do not do so at some intensity levels may be grouped together for evaluation of their response areas. We shall consider the limiting frequencies at each intensity level as defining the width of the response area at this intensity.

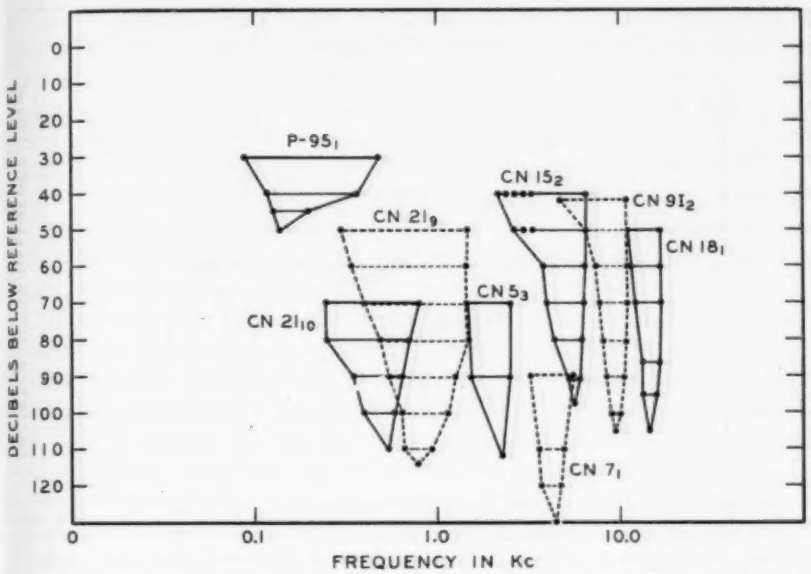

FIG. 8. Plots of responsiveness to tones at different levels of tone intensities for 8 units which show narrow response areas. Limited spread of responsiveness for tones below the best frequencies for moderately intense tones. Dots indicate limiting frequencies at a given intensity level. More than two dots on a horizontal line signify occurrence of ineffective tonal bands between the limiting frequencies.

b. Narrow and wide response areas. Individual units may differ greatly in the width and shape of their response areas. While a number of variants actually exist it is possible to assign each unit to one of the two large groups if the width of the response area to moderately intense tones is taken as a criterion. Figure 8 illustrates that for one group of units response areas remain rather narrow even if fairly intense tones are sounded. Figure 9 shows response areas for a few units of the second group. Here response areas become wide when the intensity of the tone is raised.

It is useful to take as a point of departure the best frequency of a unit and to determine separately the spread of responsiveness (in octaves) as the intensity of the tone is increased. The best frequencies of the individual units in the sample here considered ranged between 0.55 and 32.0 kc. Even though the population of best frequencies is not evenly distributed over this range and well over half of it falls between 4.0 and 16.0 kc., it is informative to calculate the average spread of responsiveness with intensity regardless of the absolute value of the best frequency. Table I presents such data for three intensity levels.

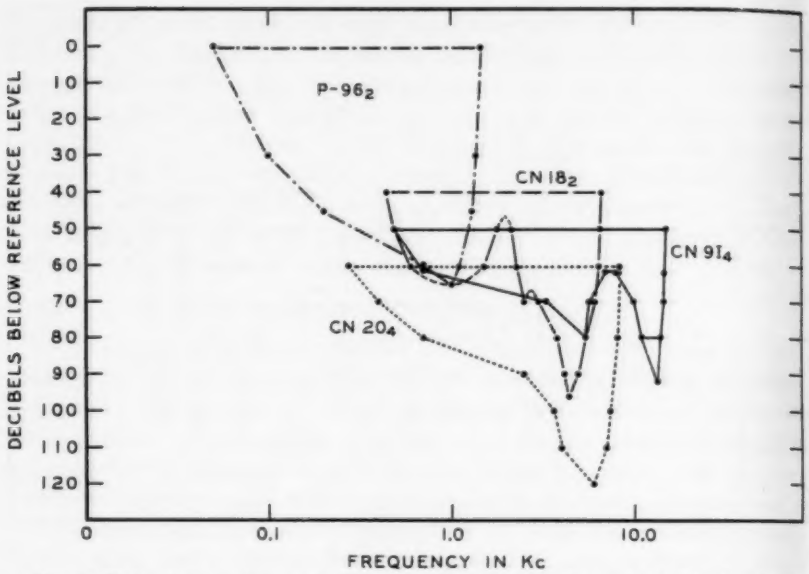

FIG. 9. Plots of responsiveness to tones at different levels of tone intensities for 4 units showing wide response areas. A broad band of tonal spectrum below the best frequency activates the units when moderately intense tones are sounded.

TABLE I

Frequencies activating the units (in octaves above and below their best frequencies) for different intensities of the tones. Figures in columns show means and standard errors. Figures in brackets show ranges of values. The indicated intensity levels represent only fair approximations since the actual determinations were not always done exactly at 20, 40 or 60 db. above the respective thresholds for the units.

	EFFECTIVE FREQUENCIES IN OCTAVES ABOVE BEST FREQUENCY			EFFECTIVE FREQUENCIES IN OCTAVES BELOW BEST FREQUENCY		
INTENSITY OF THE TONES (db ABOVE THRESHOLD)	20 db	40 db	60 db	20 db	40 db	60 db
UNITS GROUP I NO.=38	0.16∓0.02 (0.1–0.6)	0.25∓0.03 (0.1–1.1)	0.30∓0.03 (0.1–1.1)	0.29∓0.03 (0.1–0.7)	0.62∓0.06 (0.1–1.7)	1.93∓0.2 (0.4–3.8)
UNITS GROUP II NO.=37	0.17∓0.02 (0.1–0.4)	0.22∓0.02 (0.1–0.5)	0.22∓0.03 (0.1–0.5)	0.9∓0.2 (0.1–6.7)	3.50∓0.2 (1.9–6.6)	4.80∓0.3 (3.7–6.9)

It is clear that significant differences exist between the spread of responsiveness towards higher and towards lower frequencies. The spread towards higher frequencies is essentially the same for all units. It amounts to somewhat less than 0.2 of an octave at 20 db. above threshold and increases only by trivial amounts with increased intensity of the tones.

A different situation prevails in regard to spread of responsiveness towards lower frequencies. Here the intensity of the tones is critical and two modes of behavior emerge. Units in group I respond at about 40 db. above their thresholds to frequencies which are on the average no more than 0.6 of an octave lower than their best frequencies and some units of this group do not broaden their response areas by more than 0.5 an octave even at 60 db. intensity. In contrast, group II units are already activated at about 40 db. above their threshold by tones which are on the average 3.5 octaves lower than their best frequency and at 60 db. intensity level most of the tonal spectrum below their best frequency arouses responses.

It will be noted that at the lowest intensity level (20 db.) the ranges for units showing small and wide spread overlap. Some units with wide response areas already respond at this intensity to a very broad tonal spectrum, the effective tonal band for others, however, is as restricted as it is for units showing narrow response areas. Whether the latter are activated by a broad band of tones at high intensity levels (e.g., 100 db. above threshold) is unknown. It is likely that they are. This consideration, however, need not minimize the finding that individual units may differ greatly in their responsiveness to tones below their best frequencies at comparable intensity levels above their respective thresholds.

The data just presented pertain, as mentioned, to the mean spread of responsiveness regardless of the absolute value of the best frequency. Our data suggest that by doing so no noticeable error is introduced when the best frequency is about 5.0 kc. or higher. It is, however, both possible and probable that the average spread in octaves is substantially greater for low frequency units. This may be true for the spread both towards the higher as well as towards the lower frequencies. In fact, the only substantial spread towards higher frequencies in our sample occurred for low frequency elements (comp. Fig. 8). Despite the considerable interest which attaches to such findings, we shall not consider them further since our material pertaining to low frequency units is small.

3. Adaptive properties of the units

It is clear from our data that intensity of the tonal stimulus is critical for determining the initial discharge rate of a unit, the time required to approach or reach the steady state, and the final rate of discharges. Two major types of behavior are common. The most striking characteristic of the first type is that a very long time passes before the unit reaches a truly adapted state. The second type is characterized by the fact that the units quickly achieve an equilibrated rate. Other important differences regarding the discharge rate and sensitivity to stimulus intensity exist between these two basic types.

a. Adaptation with high initial discharge rate, long phase of rapid adaptation,

and a long phase of gradual decay of the discharge rate. Figure 10 illustrates adaptation curves of a unit which was studied with intensities near its threshold. The initial discharge rate varies from a few discharges at threshold to well over 200/sec., with small increments in stimulus strength. Adaptation to zero takes place within a few seconds for tones very close to threshold. For tones, however, which are only slightly more intense the adaptation curves are different. For the highest intensity tested (7 db. above threshold) there is a rapid decline in the discharge rate over a period of 15 to 20 seconds (which we shall refer to as rapid adaptation phase) followed by a very gradual decline (slow phase of adaptation). The latter is so gradual that over any short period the

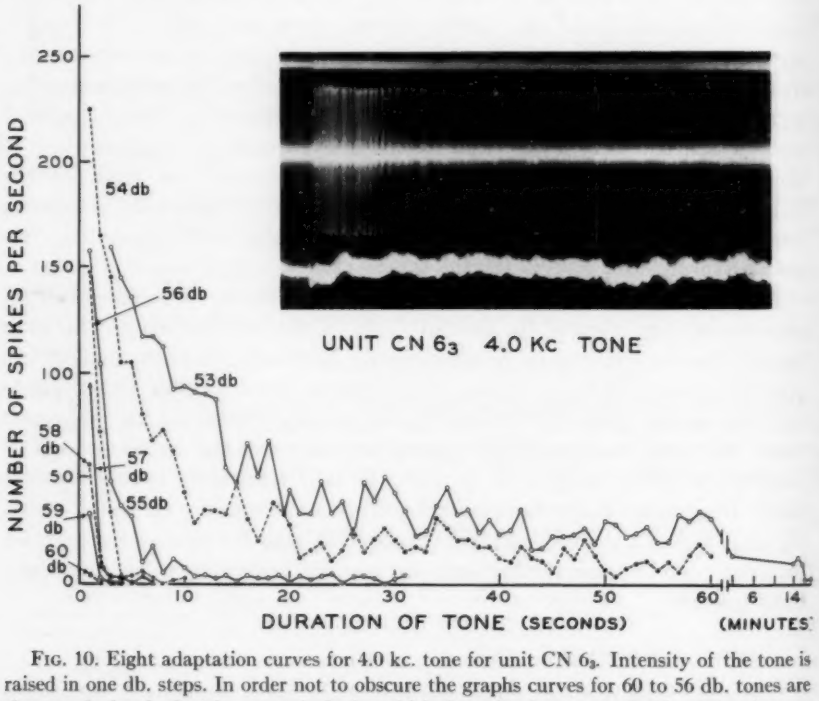

FIG. 10. Eight adaptation curves for 4.0 kc. tone for unit CN 6_3. Intensity of the tone is raised in one db. steps. In order not to obscure the graphs curves for 60 to 56 db. tones are shown only for the first few seconds during which the unit adapts to extinction. The vigorous discharges of the unit to 53 db. tone are not graphed for the first two seconds since they could not be reliably counted in the records; in the first second, however, there were substantially more than 250 spikes. Note that the unit adapts almost to extinction to a tone 7 db. above threshold but that almost 18 minutes are required to reach that stage.

The record shows the unit adapting to 4.0 kc.-57 db. tone. Middle trace: discharges of the unit; lower trace: response at the round window; upper trace: onset of stimulus and 10/sec. timing pips. Some timing pips are obscured by perforation of the film. Location: anteroventral nucleus.

discharge rate could be considered constant. That this is actually not the case becomes apparent, however, when the curve is traced over some minutes. Strictly speaking, the unit was merely approaching its adapted state (for 7 db. above threshold) for the entire time under study. The initial discharge rate (close to 250/sec.) underwent during the first 20 seconds (about 2 per cent of time under study) a decay amounting to about 85 per cent of the total drop. The remaining 15 per cent took place gradually during the slow phase of adaptation which extend over 98 per cent of the time under consideration.

Figure 11 shows adaptation curves for another unit. The adaptive behavior is similar to that shown in Figure 10. Some new facts, however, emerge. For intensities near threshold all curves are almost identical regardless of frequency used. With increased intensity all initial discharge rates increase greatly. The unit approaches its maximal discharge rate when intensity is raised by 15 to 30 db. above threshold regardless of stimulus frequency and further increments of intensity has little effect upon this rate.

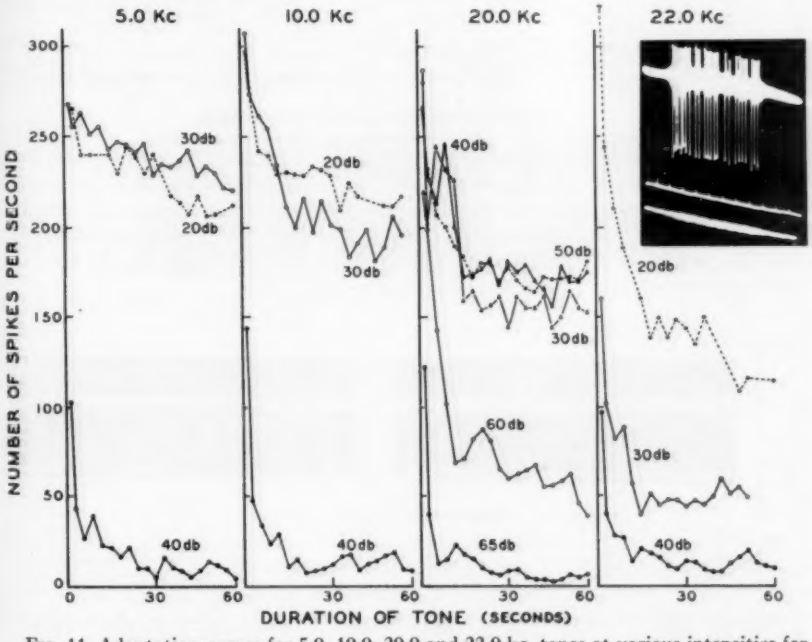

FIG. 11. Adaptation curves for 5.0, 10.0, 20.0 and 22.0 kc. tones at various intensities for unit CN 14_4. Best frequency: 20.0 kc.; threshold: 68 db. For 20.0 kc.-30 db. intensity the rate at the end of the sixth minute of continuous stimulation was about 110/sec.

The record shows the unit responding to a short pip of its best frequency at 60 db. Upper trace: discharges of the unit; middle trace: 100/sec. timing pips; lower trace: onset and duration of the stimulus. Location: posteroventral nucleus.

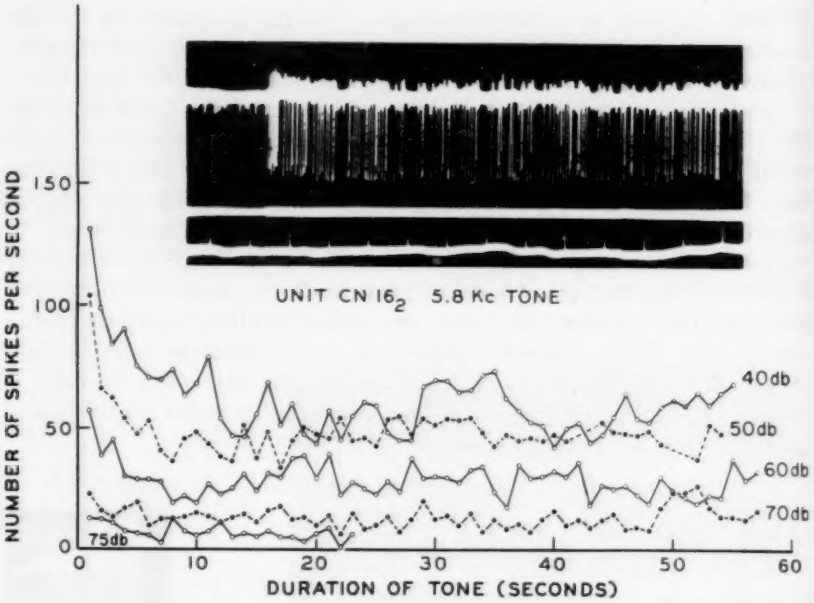

FIG. 12. Adaptation curves of unit CN 16_2 for various intensities of its best frequency tone. Best frequency: 5.8 kc.; threshold: 80 db. Note the low initial discharge rate, the sluggish reaction to intensity increments and the quick achievement of an equilibrated rate. The record shows the unit responding to 5.8 kc.-50 db. tone. Upper trace: discharges of the unit; (the large potential is denoted as unit CN 16_2); middle trace: stimulus onset and duration; lower trace: 10/sec. timing pips. Location: anteroventral nucleus.

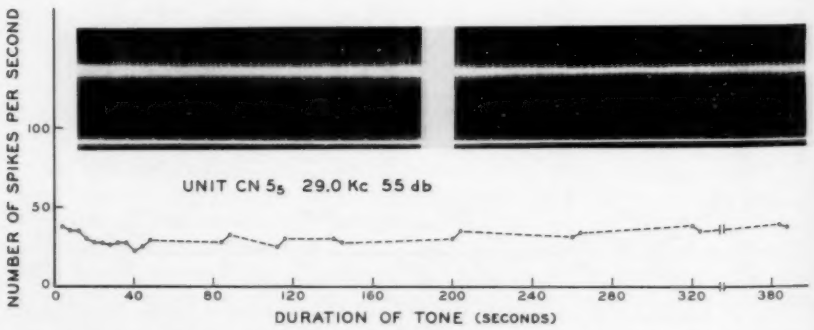

FIG. 13. Units CN 5_5 adapting to 29.0 kc.-55 db. tone. Best frequency: 29.0 kc.; threshold: 68 db. The record on the left shows the discharges of the unit at the beginning of stimulation period, the record on the right at the end of it. Upper trace: discharges of the unit; lower trace: 10/sec. timing pips. Location: posteroventral nucleus.

Adaptive behavior such as that illustrated in Figures 10 and 11 occurred in half of the 18 units extensively studied in this respect. The adaptive behavior of the remaining half was different, as will now be described.

b. Adaptation with low initial discharge rate, short phase of rapid adaptation and a steady equilibration rate. Figure 12 shows adaptation curves for a unit of this second type. Clearly this unit is sensitive to intensity but its sensitivity is by an order of magnitude smaller than that of the units shown in Figures 10 and 11. Thus, its initial discharge rate is low and rises only to about 130/sec. at an intensity 40 db. above threshold. The rapid adaptation phase is hardly noticeable near threshold and is completed quickly to the more intense tones. For all intensities the unit promptly reaches steady state values which are maintained throughout the time of observation.

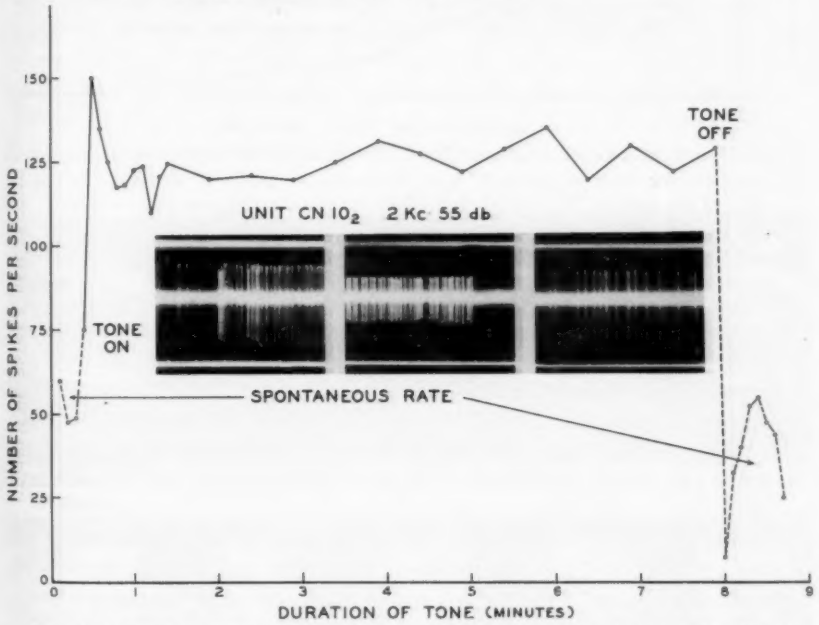

FIG. 14. Adaptation curve for unit CN 10_2 stimulated by 2.0 kc.-55 db. tone. Best frequency: 9.3 kc.; threshold: 100 db. Spontaneous discharge rate of the unit is unusually high. Record on left show discharges at the beginning of the stimulation period, record in the middle shows discharges at the end of the stimulation period, record on right illustrates spontaneous discharges a few seconds after stimulation ceased. Upper trace: onset of the stimulating tone; middle trace: discharges of the unit; lower trace: 10/sec. timing pips. Faulty synchronization of the traces displaces the beginning and the end of the stimulus to the left in respect to the middle trace. Note the diminution of spike amplitude at the end of stimulation period and gain in amplitude after cessation of stimulation. Location: dorsal cochlear nucleus.

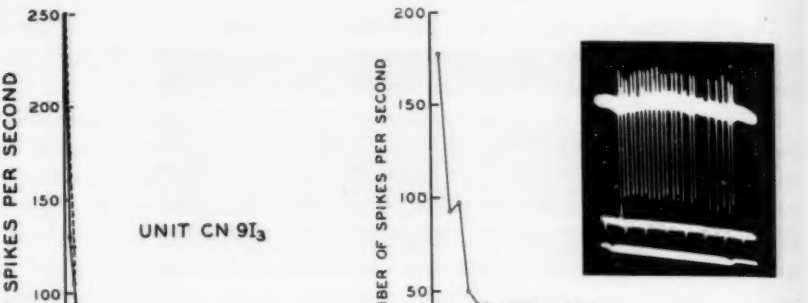
NUMBER OF SPIKES PER SECOND
UNIT CN 9I3
4.6 Kc 52 db
27.5 Kc 60 db
DURATION OF TONE (MINUTES)
NUMBER OF SPIKES PER SECOND
27.5 Kc 60db
DURATION OF TONE (SECONDS) (MINUTES)

FIG. 15. Adaptation curves for unit CN 9 I_3 showing the effect of previous stimulation on adaptive behavior. Best frequency: 27.5 kc.; threshold: 70 db. Threshold for 4.6 kc.: 62 db. Sequence of stimulation: 27.5 kc.-60 db. tone for 20 minutes (large graph); 30 seconds rest followed by restimulation with the same tone for 3.5 minutes (inset graph); 3 minutes rest followed by stimulation by 4.6 kc.-52 db. tone for 10 minutes (large graph).

Record shows the unit responding to a short pip of its best frequency tone at 60 db. Upper trace: discharges of the unit; middle trace: round window record and 100/sec. timing pips; lower trace: stimulus. Most probable location: posteroventral nucleus.

Basically this type of behavior is, with some variations, characteristic for the group. A variant of this type of adaptive behavior is shown in Figure 13. The initial discharge rate is for this unit only insignificantly higher than the discharge rate in the steady state. The latter is maintained here for at least 6 minutes.

Figure 14 illustrates another variant of this type of adaptation for a unit having a high rate of spontaneous discharges. With the onset of the tone the rate rises from about 75 to 150/sec., and then decays rapidly to about 125/sec. which is maintained without further decrement for 8 minutes of continuous stimulation.

c. Adaptation after prolonged stimulation. Figure 15 presents data on adaptive behavior of a unit before and after prolonged stimulation. A 27.5 kc.-60 db. tone was presented for 20 minutes (large graph) discontinued for 30 seconds and reapplied for 3.5 minutes (inset graph). The tone was turned off once more and 3 minutes later a tone of 4.6 kc. was applied (large graph). The differences between the two adaptation curves for 27.5 kc. are striking. The initial curve starts with a discharge rate of 250/sec. and about *15 minutes* are required

before the unit achieves a rate of 10/sec. In contrast the initial discharge rate for readaptation is but 175/sec. and the unit now reaches rates of 10/sec. already after *12 seconds* of stimulation.

It is thus clear that the immediate discharge history of a unit may have a profound effect upon its adaptive behavior. For the unit in question a 3 minutes rest was apparently sufficient for full recovery since the adaptation curve to 4.6 kc. tone is seen to be comparable in all important aspects to the original course of adaptation for 27.5 kc. It will be noted, however, that for this unit the tones were only 10 db. above threshold. We have data on other units which suggest that longer rest periods are necessary when continuous stimulation at somewhat higher intensity levels lasts no longer than one minute. We have no data on long exposures to very loud tones but it can be inferred that recovery times under such conditions are likely to be very prolonged.

It is of considerable interest to point out that the initial adaptive behavior shown in Figure 15 is in all essentials similar to that which occurs for the group of units considered under *a* above. On the other hand the behavior of the unit during readaptation (insert curve in Fig. 15) is clearly a variant of behavior discussed under *b* since an equilibrated discharge rate is established

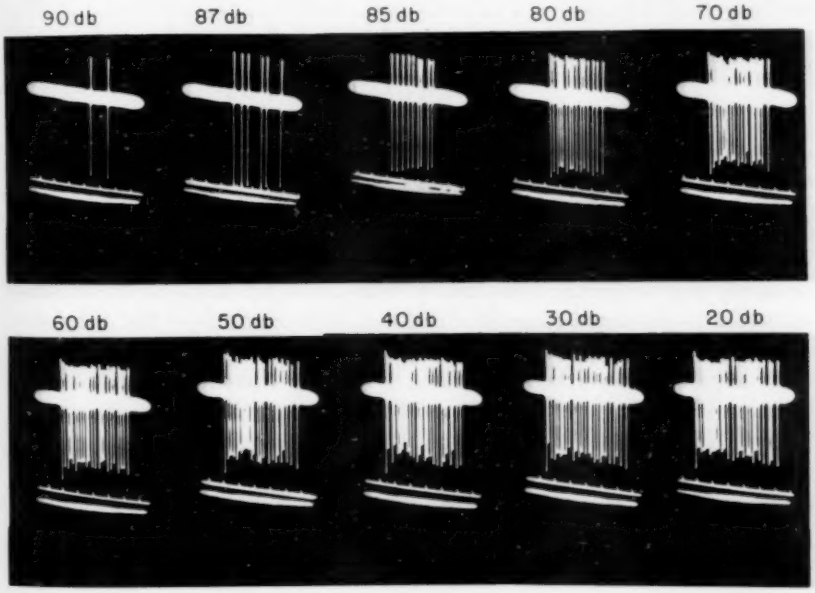

FIG. 16. Unit CN 14_3 responding to pips of 25.7 kc. tone (best frequency) at indicated intensitites of the tone. Upper trace: discharges of the unit; middle trace: record at round window and 100/sec. timing pips; lower trace: stimulus. Location: posteroventral nucleus.

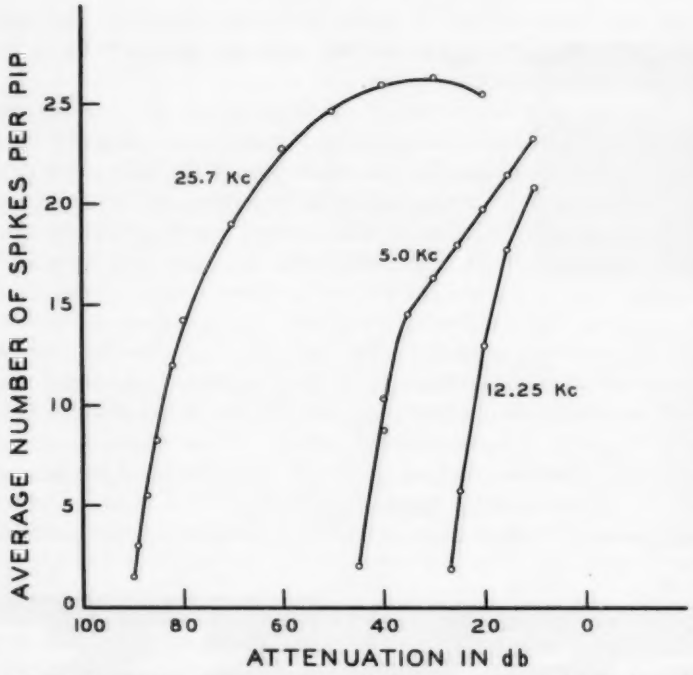

FIG. 17. Graphs relating the mean number of discharged spikes per tonal pip to intensity of the tone for 5.0, 12.25 and 25.7 kc. Unit CN 14_3 (see Fig 16). Tone pips about 50 msec. in duration. Graphs are constructed from responses to 232 pips and illustrate the common behavior of most units.

here very quickly. The data suggest then that whether a unit equilibrates fast or slowly may be merely a function of whether or not it has been previously subjected to prolonged stimulation. In fact, pips of search or test tones were almost continually employed during our experiments, and it is obvious that most units must have been subjected to prolonged stimulation before their isolation even though we guarded, as best as we could, against over-exposure.

The point to be made is that the adaptive behavior of units which show little sensitivity to stimulus strength, a low initial discharge rate, and prompt equilibration can be quite obviously interpreted as readaptation following a period of rest from prior moderately intense stimulation. Further consideration of this question appears in the discussion.

4. Suppression by excitatory tones

It is known from the work of Galambos and Davis (8) that the best frequency or any other tone which excites a unit will typically cause discharges

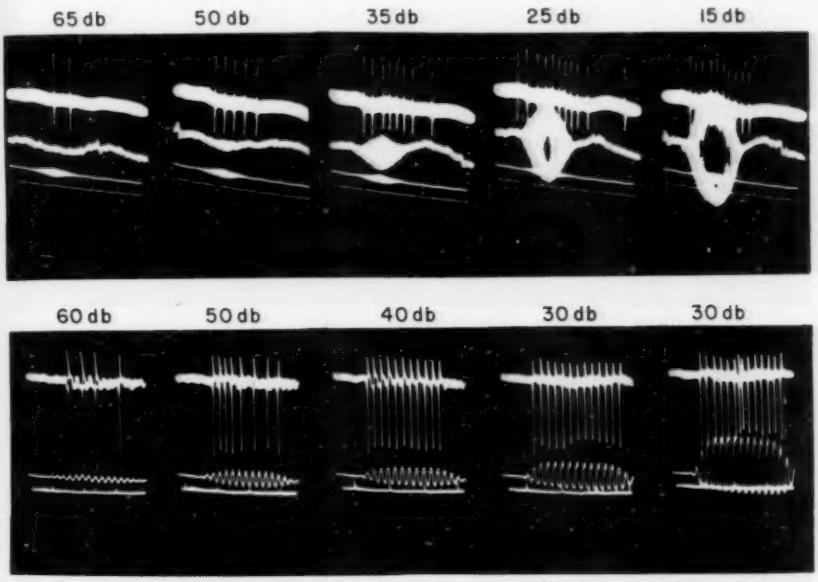

FIG. 18. Upper row: Unit CN 8_4 responding to pips of 7.0 kc. tone (best frequency: 7.8 kc.) at indicated intensities. Upper trace: discharges of the unit; 2nd trace: record at the round window; 3rd trace: stimulus; 4th trace: 100/sec. timing pips. Location: dorsal cochlear nucleus.

Lower row: Unit CN 7_2 responding to pips of 0.5 kc. tone (best frequency: 3.5 kc.) at indicated intensities. Upper trace: discharges of the unit; 2nd trace: record at the round window; lower trace: 100/sec. timing pips. Gain for the spike reduced for the second and consecutive records. Gain of the round window record reduced between the 2nd and 3rd records. The last record shows the round window trace at the same gain as in records 1 and 2. Note that at some intensities a discharge follows each cycle. Note also that this relation is by no means invariant. Location: posteroventral nucleus.

of an increasingly larger number of spikes per tonal pip when intensity is increased. With intense stimuli a discharge plateau is usually reached. Figure 16 illustrates such a common behavior for a high frequency element and in Figure 17 the average number of spikes per pip is plotted in relation to tone intensity for three frequencies activating this unit. Figure 18 shows similar behavior of two other units responding to low frequencies.

This well known behavior is typical but not universal in our material. The response of some units to increase in intensity of its best frequency is different. Figure 19 illustrates a unit of this type. Here the unit responds at first to increase in intensity in the usual manner as far as the number of spikes is concerned. As the intensity is further increased, however, the number of discharges

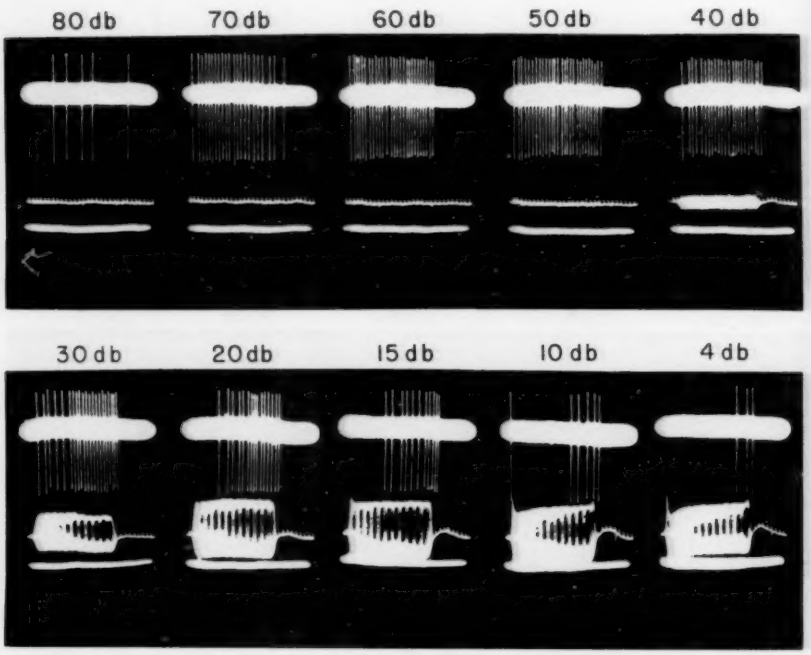

FIG. 19. Unit CN 15_2 responding to pips of 5.3 kc. tone (best frequency) at indicated ntensities of the tone. Strong tonal pips produce fewer spikes than do weak stimuli. Note the iong latencies and the build up of the discharge rate to more intense pips. Upper trace: lesponses of the unit; middle trace: round window record and 100/sec. timing pips; lower trace: stimulus. Location: dorsal cochlear nucleus.

diminishes rather than increases and intense tonal pips may be almost ineffective in evoking discharges. Figure 20 plots similar data for two other units.

We shall present in some detail our observations on unit CN 15_2 since this element was the one most thoroughly studied among the units of this type in our material.

Figure 21 provides evidence that the effect is not frequency specific since diminution in number of spikes per pip with increased intensity occurs for the best frequency (5.3 kc.) as well as for all other excitatory frequencies tested. For 2.3 kc. the entire curve is shifted markedly towards intense stimuli. The curve for 4.5 kc. is of special interest. Intensities up to 40 db. below reference level aroused discharges but none occurred with intensities of 31 and 30 db. With further increase in intensity the tone is again effective. It should be mentioned that the size of the aural microphonics reached a peak with tone

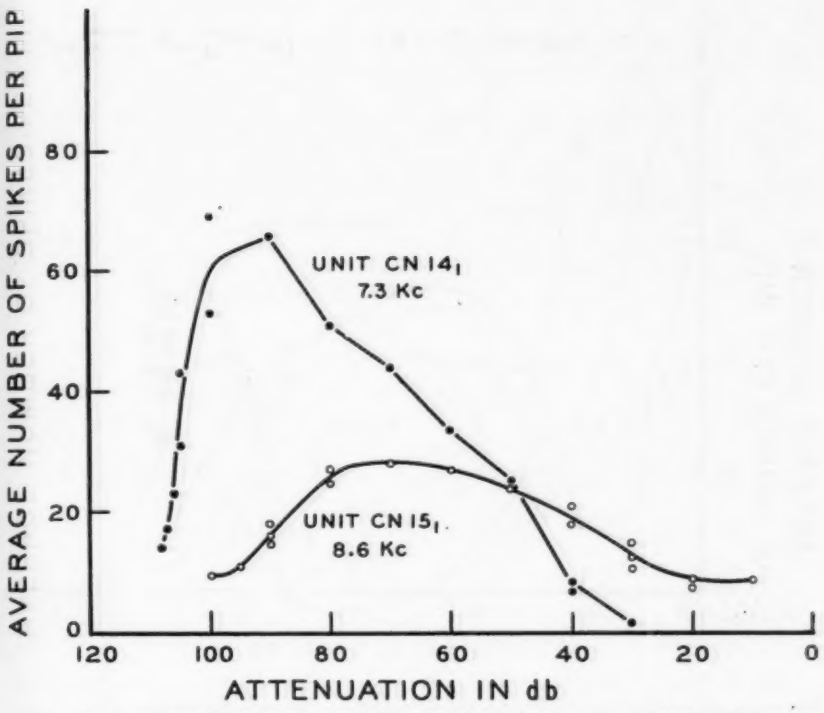

FIG. 20. Graphs relating the mean number of discharged spikes per tonal pip to intensity of the tone for two other units for which intense tones tended to suppress discharges. Unit CN 14$_1$. Best frequency: 7.3 kc. Duration of pips: about 300 msec. Unit CN 15$_1$. Best frequency: 8.2 kc.; duration of pips: about 200 msec. Graphs are constructed from responses to 194 pips. Locations for both units: dorsal cochlear nucleus.

intensities around 30 db. also and was distinctly smaller for 20 and 10 db. pips. Whatever this may mean it is evident that the 4.5 kc. tone pip near 30 db. was completely unable to produce neural activity. All other frequencies, however, also proved ineffective, albeit at somewhat higher intensities.

The effect discussed is apparently inhibitory in nature. This is suggested by latency considerations and apparent from the fact that an intense tonal pip which does not elicit discharges actually eliminates those which are provoked by another adequate stimulus. Since vigorous discharges will commence if an intense stimulus is sounded for a long enough time the effect can be shown to be but a temporary one. We shall now consider the evidence for these statements.

a. Latency of the discharge train. Records in Figures 16 and 18 indicate that a typical unit responds promptly after onset of the tone. With higher in-

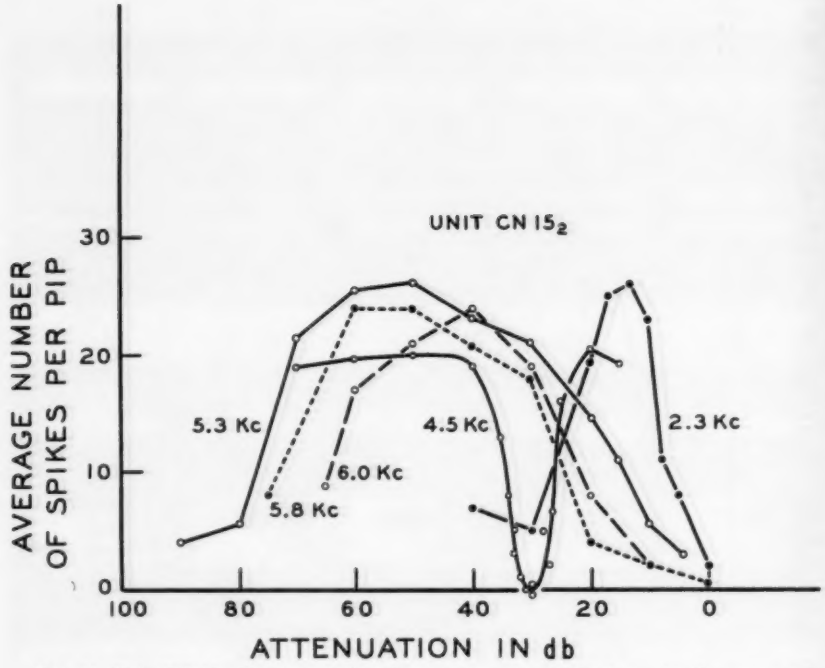

FIG. 21. Unit CN 15_2. Graphs relating the mean number of discharged spikes per tonal pip to intensity of the tone for 2.3, 4.5, 5.3, 5.8 and 6.0 kc. Duration of tonal pips is the same (about 200 msec.) for all determinations. Graphs based on responses to 312 pips.

tensities the frequency of the discharge train either diminishes or remains at best steady as the tone continues. By contrast the latency to the burst in Figure 19 is long and the interspike intervals tend to decrease with the duration of the tone. The discharge train is being "built up". It is convenient to take for the units of this type as a measure of the delay the latency to the second spike in the train. Such measurements pertaining to data shown in Figures 19 and 21 are plotted in Figure 22. The latency to the second spike is long, it decreases at first with increase of tone intensity, to increase again quite remarkably with further increase in intensity.

b. Suppression of discharges provoked by a stimulating tone. It is of considerable interest that intense stimuli that fail to evoke discharges (Figure 21) may suppress activity aroused by another adequate stimulus. Thus in Figure 23 unit CN 15_2 is driven by its best frequency (5.3 kc.-40 db.). Three pips of 4.5 kc. tone at 30 db. are sounded. Each pip suppresses the discharges completely; the inhibition outlasts the duration of the pip by about 400 msec. and thereafter the discharge rate builds up slowly to the original rate of discharges.

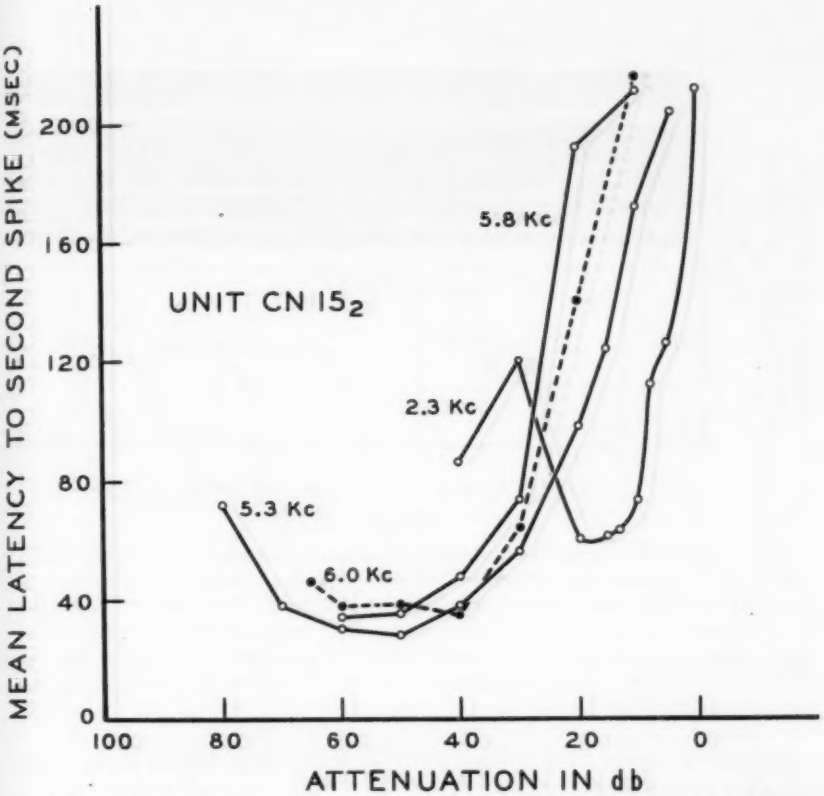

FIG. 22. Unit CN 15_2. Graphs relating the mean latencies to the second spike in the response train to intensity of the tone for 2.3, 5.3, 5.8 and 6.0 kc. tone pips. In order to simplify the graphs values for 4.5 kc., which follow the same pattern, are not shown.

c. Adaptation of "build up" units. If a unit of the type considered is stimulated by continuously sounding tones rather than by short pips remarkable adaptation curves result. Figure 24 shows such curves for unit CN 15_2 for its best frequency (5.3 kc.) at different intensities. In contrast to the usual adaptation curves (compare Figs. 10 to 15) discharge rate *increases* during the first second (except for the weakest intensity) and this phenomenon becomes the more striking the more intense is the tone. Inset curves of Figure 24 analyse the discharge rates at intervals of 100 msec. showing the initial suppression of all discharges and the gradual build up of the rate after onset of an intense tone. For comparison a drop of discharge rate during the first two seconds is shown for another unit (CN 6_3) displaying the usual adaptive behavior.

Apart from the initial build up, the adaptation curves shown in Figure 24

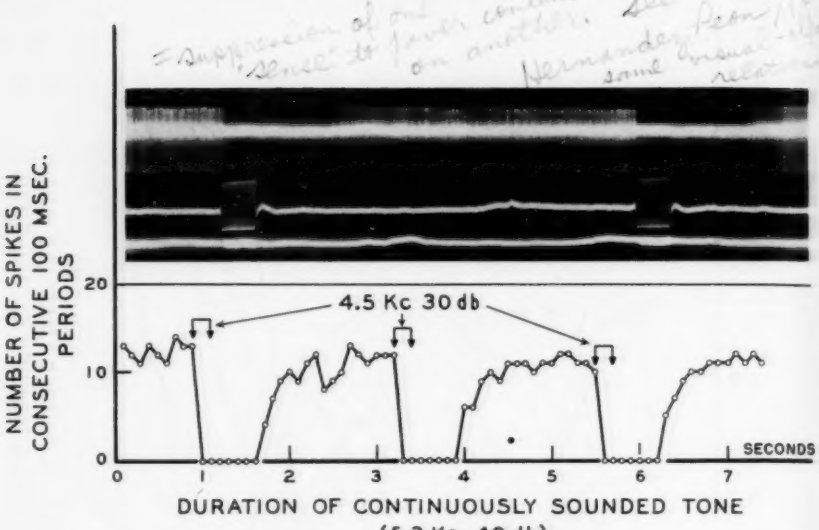

FIG. 23. Unit CN 15_2 driven by continuously sounded best frequency tone (5.3 kc.-40 db.) Pips of 4.5 kc.-30 db. tone (200 msec. in duration) sounded intermittently suppress discharges. Graph plots the number of spikes in 0.1 sec. intervals. Note complete suppression of all discharges for about 400 msec. after 4.5 kc. tone ceased and the slow build up of the discharge rate to the original level. The record shows the effect of two such pips. Upper trace: discharges of the unit; middle trace: record at the round window and 10/sec. timing pips; lower trace: stimulus.

are similar to those which are generally shown by units which are but little sensitive to tone intensity.

All units for which intense pips of their best frequencies were apparently inhibitory displayed long latencies and "build up" characteristics of the discharge train. We have encountered six such units in our sample of 30 in which intensity-frequency relationships were studied extensively. However, a few units which showed longer latencies and "build up" characteristics did not clearly respond with fewer spikes to strong stimuli. Whether all "build up" units belong to the same group and whether all display special inhibitory features must be left unanswered at present.

5. Response to clicks

It is well known that a click stimulus produces some kind of a "slow wave" response throughout virtually the entire extent of any synaptic region in the auditory system. This response which consists of a series of deflections varies appreciably in shape, size, and relative prominence of the components in the various segments of the cochlear nuclear complex. However, we shall not

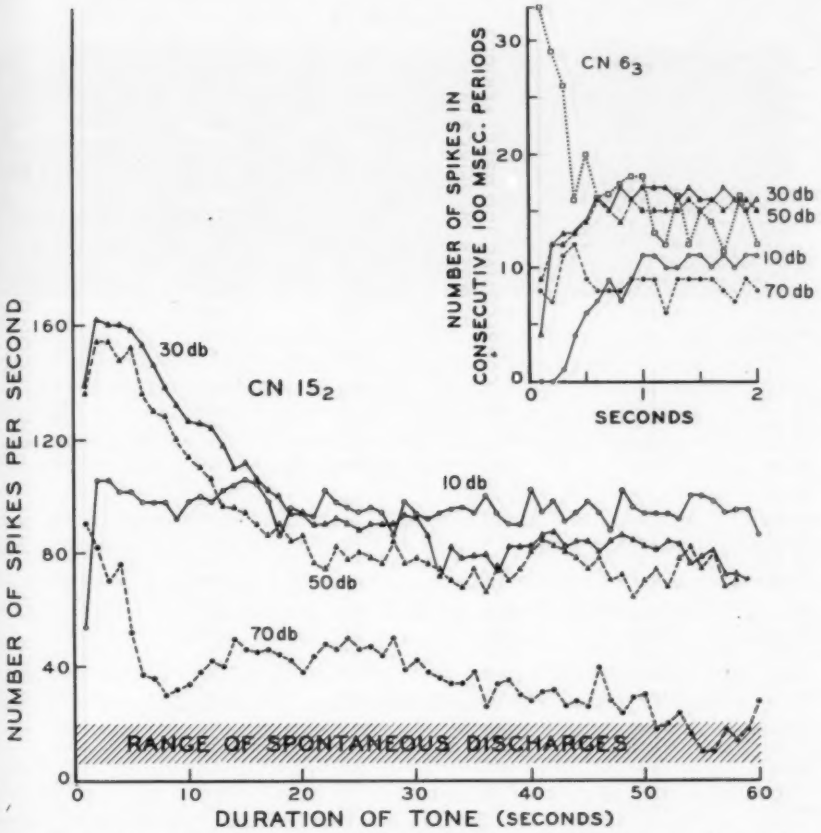

FIG. 24. Adaptation curves for unit CN 15_2 for 5.3 kc. tone (best frequency) at various intensities. For intense stimuli the number of spikes in the first second is markedly lower than in the second second. Inset curves graph the number of discharges in the first two seconds at 0.1 sec. intervals. Note the period of suppression and the gradual build up of the discharge rate. For comparison a typical decline in the discharge rate is exemplified by unit CN 6_3 (see also Fig. 10) which adapts here to an 8.0 kc.-60 db. tone. Response area for unit CN 15_2 for weaker tones is shown in Figure 8. Response area for intense stimuli is not plotted there since the responsiveness of the unit for such tones depends not only on the intensity and frequency of the tone but also on its duration.

analyse here the slow wave activity but we shall consider only the responses of the units to clicks.

a. Sensitivity. Only a small fraction of the units studied was highly sensitive to click stimuli and many units could not be aroused even by loud clicks despite the ubiquity of the slow wave response to such stimuli. If a unit responded both to tones and clicks its threshold for tone was almost invariably lower than

TABLE II

Data for 35 units pertaining to their best frequencies and thresholds to tones and clicks (first to third column of figures). The fourth column shows threshold intensity for clicks (first neural response) as recorded at the round window (RW) with a gross electrode. Last column of figures shows the difference between threshold to clicks of an individual unit and the click threshold at the round window in a given experimental animal.

UNIT	BEST FREQUENCY (Kc)	THRESHOLD OF THE UNIT		THRESHOLD TO CLICK AT RW (db)	DIFFERENCE (db)
		TO TONE (db)	TO CLICK (db)		
CN 7_2	3.5	116	101	115	14
CN 16_3	3.5	58	32	80	48
CN 21_5	3.5	100	88	105	17
CN 18_2	4.4	96	48	65	17
CN $9II_4$	4.5	126	102	110	8
CN 21_4	5.0	115	100	105	5
CN 16_2	5.8	80	46	80	34
CN 20_4	6.0	120	110	105	-5
CN 15_0	7.8	98	60	90	30
CN 5_1	8.0	136	100	100	0
CN 21_3	8.0	100	84	105	21
CN 10_1	8.2	110	85	105	20
CN 12_1	8.4	128	100	117	17
CN 14_8	8.8	78	48	95	47
CN $9I_2$	9.3	105	30	110	80
CN 10_2	9.3	100	84	105	21
CN $9I_{30}$	9.6	122	90	110	20
CN $9I_1$	9.8	122	95	110	15
CN 12_5	10.3	90	55	117	62
CN 17_4	12.8	65	55	95	40
CN $9I_4$	13.5	92	75	110	35
CN 21_2	14.0	87	65	105	40
CN 12_3	14.8	88	50	117	67
CN 20_3	15.5	110	85	105	20
CN 17_3	16.0	50	40	95	55
CN 13_9	16.5	90	54	105	51
CN $9II_3$	18.3	85	55	110	55
CN 14_4	20.0	68	37	95	58
CN $9II_1$	21.0	90	51	110	59
CN 20_2	23.5	108	70	105	35
CN 14_3	25.7	91	34	95	61
CN $9I_3$	27.5	70	50	110	60
CN 14_{20}	28.0	86	31	95	64
CN 14_2	28.5	60	32	95	63
CN 19_2	32.0	38	45	100	55

that for clicks as shown in Table II where the click threshold as recorded at the round window is also tabulated. As shown in Table II the thresholds for tones are satisfactorily low for most units. The apparently higher thresholds for higher frequencies can be expected because of a substantially lower output of out stimulating system for such tones (see Methods). A relation is present between the best frequency of a unit and its sensitivity to clicks if this sensi-

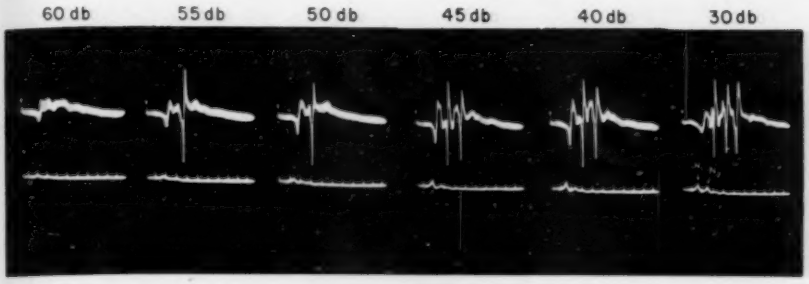

FIG. 25. Unit CN 12_8 (best frequency 10.3 kc.-90 db.) responding to increasingly stronger clicks. Location: anteroventral nucleus. Upper trace: response recorded by microelectrode (stimulus artefact at extreme left); lower trace: record at the round window and 1000/sec. timing pips. N_1 and N_2 denote respectively the first and second neural elevations. Note the configuration of the slow wave response, the repetitive discharges of the unit to stronger clicks and the concomitant shortening of the latent period.

tivity is measured by the difference between the click threshold at the round window and the individual click threshold of the unit. This difference is substantially smaller than 30 db. for 13 out of 18 units whose best frequencies are lower than 10.0 kc. By contrast, it is with one exception substantially higher than 30 db. for all higher frequency elements. The data suggest strongly that a low frequency element is likely to be much more sensitive to clicks than a high frequency unit. How to interpret the exceptions which occur is not at all clear.

As is the case in other synaptic regions of the auditory system, if the unit responds to clicks, the security of the response, the latency, and the number of discharges are all parameters of stimulus intensity.

b. Security of the response. Figure 25 shows a series of responses to clicks at different intensities. Figure 26 plots the pertinent data for another unit. Figure 26 demonstrates that percentage of clicks evoking spikes rises smoothly from less than 10 per cent to 100 per cent with an increment of 7 db. The increment in intensity required to increase the responsiveness from near zero to 100 per cent varies appreciably for different units. Values around 10 db. are most frequent. The most sensitive units in this respect required only 4 db. while on occasions over 20 db. was required (Fig. 27).

c. Number of spikes per response. When intensity is increased a unit may continue to discharge one spike per click but more frequently trains of 2, 3 or 4 spikes appear (Figs. 25 and 26). Usually, but not invariably, a 2-spike train is in evidence only after the unit reached a 100 per cent responsiveness level. As could be expected if a unit is relatively insensitive to increments in click intensity (once the threshold has been reached) it is likely to remain a one

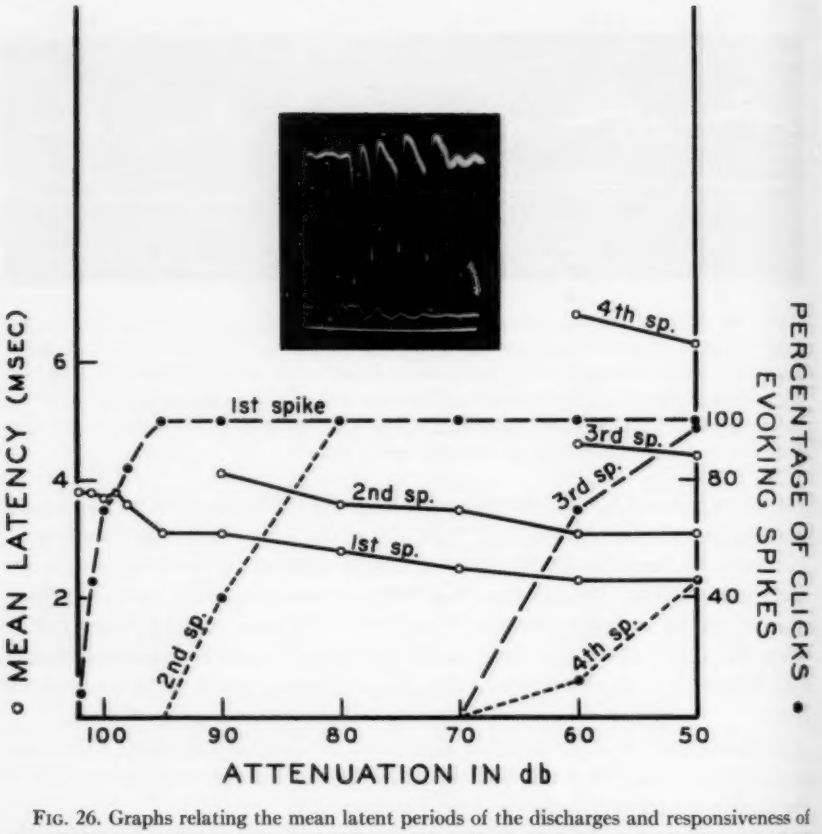

FIG. 26. Graphs relating the mean latent periods of the discharges and responsiveness of the unit (measured by percentage of clicks evoking spikes and the number of spikes per click) to intensity of clicks. Unit CN 7_2. Best frequency: 3.5 kc.; threshold 116 db. Graphs constructed from 356 responses. The record shows the unit responding four times to a 50 db. click. Upper trace: discharges of the unit; middle trace: record at the round window; lower trace: 1000/sec. timing pips. Location: posteroventral nucleus.

spike response over a large span of intensities. However, a fast rise of responsiveness does not assure a repetitive response particularly if the unit has long latent period (see below).

d. Latency to spike. Latency-intensity relationship typical for the majority of units is illustrated in Figure 26. The curves for the second and successive spikes rather closely parallel that of the first. The mean latency to the first spike is longest near threshold and shortens as intensity increases. This relationship held true for all 20 units in which this relationship was studied. The mean latency shortens progressively over considerable intensity range. While the

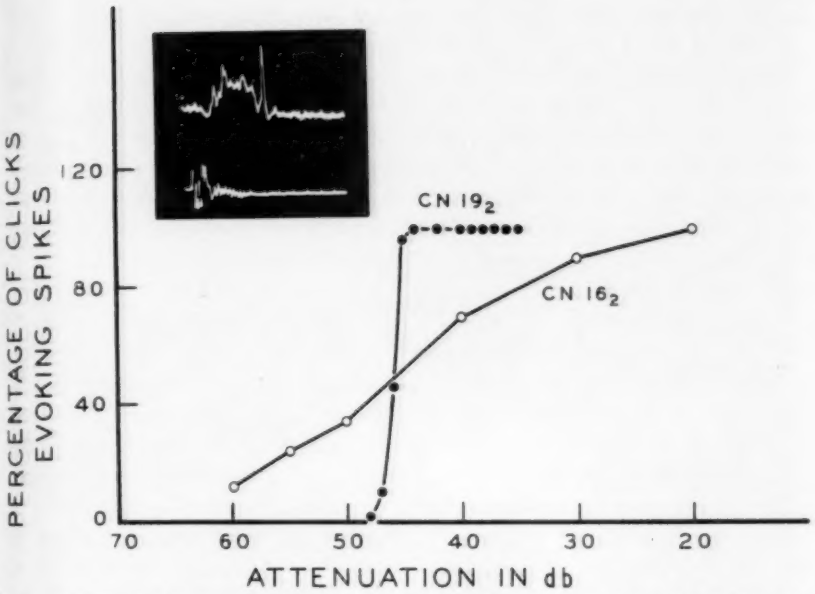

FIG. 27. Graphs relating the percentage of clicks evoking spikes to intensity of clicks for two units and illustrating the range of variability in responsiveness. Discharges of unit CN 16_2 (best frequency: 5.8 kc.) are shown in Figure 12. Location: anteroventral nucleus. Graph is based on 300 responses.

Graph for unit CN 19_2 is based on 497 responses. Location: dorsal cochlear nucleus. Record shows unit CN 19_2 discharging to a 44 db. click. Upper trace: slow wave response and the discharge of the unit; lower trace: record at the round window and 1000/sec. timing pips. Best frequency of the unit was 32.0 kc.

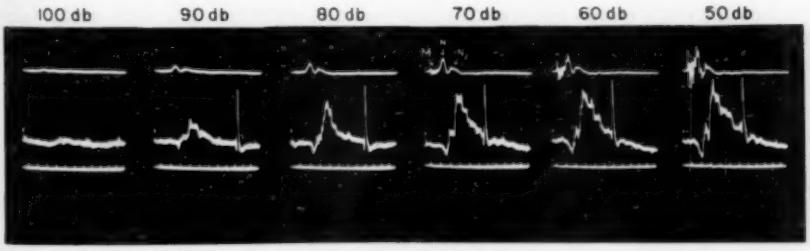

FIG. 28. Unit 21_3 responding to clicks at indicated intensities. Best frequency: 8.0 kc.; threshold: 100 db. Upper trace: record at the round window. M—aural microphonic potentials, N_1 and N_2—first and second neural responses; middle trace: slow wave response to clicks and discharge of the unit; lower trace: 1000/sec. timing pips. The unit discharged always but one spike per click and the latent period is relatively very long even to strong stimuli. Location of the unit uncertain: either dorsal cochlear or anteroventral nucleus.

absolute latency values differed for different elements the discharge of the unit typically occurred in conjunction with the slow wave, usually near its peak.

A few units had latencies substantially longer than those shown in Figures 25 and 26. Two of these are shown in Figures 27 and 28. In Figure 28 the latency near threshold is about 8.0 msec. and shortens to strong clicks to about 6.0 msec. It will be noted that the unit fires after the slow wave response is substantially over. It would appear that in relation to the slow wave a response to click may be "early" or "late" in the cochlear complex as it is known to be true in other synaptic regions of the auditory system.

DISCUSSION

Tonotopical Localization

The demonstration that each major division of the cochlear complex is tonotopically organized and that each division is likely to command a full tonal spectrum raises questions as to the role each of them may play in the auditory performance of the animal and as to the anatomical means by which such an organization is achieved.

Our experiments shed no immediate light on the functional significance of the separate divisions. While it seems unwise to stress negative findings, it may be stated that at least in deeply anesthetized animals and as far as excitatory phenomena are concerned it has not proved possible thus far to identify a given unit as belonging to a given division by virtue of its discharge characteristics. This was true even though such characteristics are by no means uniform for all units. Whether such findings should be taken to imply that the expected differences will become apparent if behavior of cell populations rather than of single neurons is studied or whether some significant differences between individual neurons were either missed in our analysis or eliminated by the deep anesthesia employed must remain an open question. For some inhibitory phenomena a correlation does exist since all units inhibited by their own best frequencies at high intensity levels were located in the dorsal cochlear nucleus. In our sample this observation is significant at 1 per cent confidence level.

By contrast the morphological arrangement resulting in the tonotopical organization appears easier to comprehend. A scheme which could be used to interpret our experimental observations is shown in Figure 29. The scheme intends to account for the high-low sequences in each diyision and for the abrupt jumps in best frequencies as the electrode leaves one division and enters the next. The data do not permit to suggest any details as to the actual course and distribution of the fibers. The scheme is based on the classical findings (2, 11, 13) and on observations of Lorente de Nó, and Levy and Kobrak (15 to 17). Each cochlear fiber is thus assumed to bifurcate into a branch

FIG. 29. Schematic distribution of cochlear fibers within the cochlear nuclear complex which could account for topical organization of frequency sensitive elements. An orderly arrangement of fibers from high to low is assumed to exist in a dorsoventral sequence. Two fibers are drawn. Fiber 1 derives from the middle turn of the cochlea; fiber 2 from a region more basalwards. Each fiber distributes to all three nuclei. The scheme aims to account for the orderly sequences of best frequencies in each division and for the jumps in best frequencies as the electrode leaves one division and enters the next. The data at hand are inadequate for deductions as to the detailed distribution of the fibers within each division and the distribution actually drawn is but one of a number of possible variants.

a = cut edge of the restiform body and the superior cerebellar peduncle, b = cut edge of the brachium pontis, Av = anteroventral nucleus, Pv = posteroventral nucleus, Dc = dorsal cochlear nucleus. See also Figure 1. For clarity sake the posteroventral nucleus has been drawn in schematically as a narrow cone.

ramifying in the anteroventral nucleus and another which supplies the posteroventral and dorsal cochlear divisions. Furthermore, the fibers from the apical coil are assumed to distribute in the ventrolateral portions of each division while those arising in the middle and basal turns lie successively more dorsomedially.

Our separation of the ventral cochlear nucleus into anteroventral and a posteroventral division and the presumption that each represents a distinct functional unit agrees very well with the findings of Cajal (2) who pictured terminations in the anteroventral nucleus as consisting of endings of Held, while those in the posteroventral nucleus are drawn by him as rich nests of ramifications. The only part of the scheme which does not have substantial anatomical support is the presumed course and terminations of fibers in the dorsal cochlear nucleus. While the available information is scanty the findings of Lorente de Nó (16) imply that the fibers there ramify along their ventrodorsal course and some doubts have been expressed (20, 24) as to how many direct fibers from the cochlea actually terminate in the dorsal cochlear nucleus.

Our findings that a precise tonotopical organization exists in the cochlear complex is at variance with recent findings of Katsuki *et al.* (12) who failed to find evidence for it.

Response Areas

The observation that all units respond to a limited range of tones *above* their best frequency was made some time ago (8) and has since been amply confirmed (12, 25). Our data provide some new quantitative information. With the possible exception of low frequency elements the spread towards higher frequencies amounts to about 0.2 of an octave and is but little increased by intense tones. Schuknecht's data (23) suggest that each octave in a cat is represented over a distance of ca 2.2 mm. of the basilar membrane. If one assumes that the spread of responsiveness reflects the excursion of the basilar membrane it follows that the *functionally effective* displacement of this membrane from the point of the maximal excursion towards the apex occurs over a distance which is about 0.5 mm. even if fairly loud tones are sounded.

In respect to frequencies lower than the best our material shows a bimodal distribution. There is little doubt that some units display fairly narrow and others very broad response areas. These findings harmonize some divergent views (8, 12, 25) as to the actual shape of the response areas in the cochlear complex. To what extent inhibitory effects may be instrumental in shaping the response areas is uncertain. There is no doubt, of course, that when inhibitory effects are demonstrable they do affect it. On the other hand a response area of a unit may be often determined without any inhibitory activity being apparent.

There is fair agreement on the basis of anatomical studies that there are some cochlear fibers which innervate only a few hair cells and others which supply a great number of them over relatively long distance of the cochlear spiral (11, 14, 18). One is inclined therefore to consider these anatomical arrangements as possibly responsible as well for wider and narrower response

areas. If this were so, the narrow and wide response areas could be in general expected to reflect connections with the inner and outer hair cells respectively. But only future work can decide whether this consideration has merit.

Adaptive Behavior of Single Units

As shown by our data it is injudicious to make statements about the adaptive behavior of single elements unless stimulus strength is specified. For not only the initial discharge rate but also the course of adaptation and the final rate are critically dependent on the intensity of the tone. The effect of intensity is most pronounced for values near threshold and seems to reach a maximum at about 20 to 40 db. above it. Two types of adaptive behavior have been demonstrated. The first is characterized by an exquisite sensitivity of the unit to small changes of tone intensity, by high initial discharge rate, and by the long time which is required before a stable rate is established. Except for intensities near threshold units of this type do not reach a fully adapted state for about 15 to 20 minutes of continuous stimulation. Units of the second type are sensitive only to relatively large shifts in stimulus strength. The initial discharge rate is low, and a steady discharge rate is reached quickly and maintained without demonstrable change for a long time.

It seems reasonable to assume that all the features of the behavior of the second type may develop from the respective characteristics of the behavior of the first type. It has been moreover argued (see p. 231) that the first type of behavior can be actually converted into the second provided the unit is stimulated long enough. We shall therefore assume in further discussion that all units, if rested, display similar adaptive behavior and the differences noted are primarily the consequence of previous exposure to sounds. We suggest than that the behavior of the first type discloses the initial course of adaptation while the behavior of the second type and its variants manifests phenomena of readaptation of units already adapted to various degrees.

It is evident from our data that the adaptive process requires a much longer time than hitherto believed (8). This time is a function of both intensity and duration of the tone. A rested unit activated by very moderate intensities may require 15 to 20 minutes before its adapted rate is actually established. Adaptation seems to impair greatly the capacity to respond to small increments in stimulus intensity, a fully adapted unit being less sensitive than an unadapted one by as much as an order of magnitude of intensity. Recovery after exposure seems again to be a function of duration and strength of the stimulus which produced the adaptation. For stimuli of short duration near threshold the recovery time merely requires seconds; minutes are needed after moderate tones of short duration; and presumably hours would be necessary after loud tones were sounded for a long time.

If these considerations are valid it follows that stimulation of units at various stages of recovery must frequently occur under physiological conditions. It seems possible that readaptation of partly adapted units is a common mechanism operating in the act of hearing. It is of interest to point out that certain psycho-physical observations concerning adaptation or the so-called auditory fatigue in man (e.g., the time course of the threshold elevation from levels of stimulation only slightly above threshold) appear predictable from the adaptive behavior of single elements.

Suppression By a Loud Best Frequency Tone

A variety of inhibitory phenomena are known to occur in the cochlear complex (6). Thus the best frequency may abolish or diminish spontaneous discharges of a unit at intensities below the threshold for excitation. Spontaneous discharges may be also inhibited by tones which are either higher or lower or higher and lower than are those which excite. In addition it has been shown (6) that a discharge produced by a stimulating tone may be abolished if another tone or noise is presented simultaneously. To this assembly of data an observation (true for 20 per cent of our units) may be added, namely, that the best frequency (or other excitatory tone) presented as intense tonal pips may for a time suppress discharges of the very unit it excites optimally (or well) if the intensity is weak. This observation seems concordant with those in the medial geniculate (9) and auditory cortex (4) where some units were observed to respond invariably to weak clicks but not at all to intense ones.

Whether suppression caused by loud tones is a bona fide physiological phenomenon, and what its role could be in the act of hearing must be left an open question at present. It could be that the effect is produced by activation of portions of the basilar membrane which surround the segment of it which when moved alone causes excitation. Inhibition of this kind could be expected to be frequency non-specific and all tones which excite should be also inhibitory at some intensity. From considerations of Békésy curves (1) pertaining to movements of the basilar membrane and from data discussed on page 246 one could speculate that the most effective inhibitory tones could well be those which are lower than the best frequency by about 0.2 of an octave. Curves in Figure 21 are in general agreement with such notions but more data are needed before one can evaluate with confidence the significance of such findings.

Response to Clicks

The presented data establish that units of various best frequencies may respond to clicks. Units highly sensitive to click are usually low frequency elements while high frequency units have, as a rule, high click threshold. However, a low best frequency of a unit does not assure a low threshold for clicks. Whether these facts should be taken to imply that the effective dis-

turbance in the cochlea produced by clicks is limited to frequency region below 10.0 kc. is uncertain.

A response of a unit to a click tends to become repetitive with increased intensity of the stimulus. This behavior is common in all synaptic regions of the auditory system (4, 9, 21) and indeed it is typical for other great afferent systems as well. With increasing intensity the latency to the discharge shortens and the minimal values are of the order of 2.0 to 3.0 msec. These times are reasonable considering the minimal latencies for units in the medial geniculate and auditory cortex (4, 9). Interestingly, but not unexpectedly some units discharge "late". The longest latent periods in our sample were 6.0 to 8.0 msec. in response to strong clicks. These late discharges may activate some of the units which respond late in the medial geniculate and auditory cortex (4, 9). It is of interest that a latency of 6.0 to 8.0 msec. in the cochlear nuclei implies that at a time when some units fire here for the first time many cortical auditory units are already discharging to the same stimulus. Clearly some neurons do not serve merely as simple relays even in the first synaptic regions of the auditory system.

SUMMARY

1. On the basis of cytoarchitectonic findings three major subdivisions are recognized in the cochlear nuclear complex of the cat. They are: the anteroventral nucleus, the posteroventral nucleus, and the dorsal cochlear nucleus. Evidence is presented that in each of these nuclei there exists a tonotopical organization such that the frequency sensitive neurons are arranged from high to low in an essentially dorsoventral sequence.

2. The response areas of all units show a sharp high frequency cut off. The average spread of responsiveness does not extend, for tones up to 60 db. above threshold, over more than about 0.3 of an octave *above* the best frequency. The spread of responsiveness towards lower frequencies varies. Units with narrow response areas respond to tones 60 db. above threshold to frequencies which are lower by 0.4 to 3.8 octaves *below* their best frequencies. At comparable intensities units with wide response areas can be activated by almost all tones lower than their best frequencies.

3. The adaptive behavior of units to tones are described. Intensity of the tone is critical for the course of adaptation. Two principal modes of adaptive behavior have been observed. The first is characterized by high sensitivity to small differences in tone intensity, by high initial discharge rate, and by long times required before a unit reaches a steady discharge rate. The second mode of behavior is characterized by low sensitivity to stimulus strength, by relatively low initial discharge rate, and by quick attainment of the equilibrated rate which is maintained without change for a long time. It is suggested that these two modes of behavior represent respectively, the adaptation of a fully

rested unit and the readaptation of a unit previously adapted to tonal stimuli. It is probable that both adaptation and recovery time require many minutes even for moderate exposures.

4. It is shown that the well known relationship between stimulus intensity and discharge rate is not universally valid. For about 20 per cent of all units the best frequency and other excitatory tones, if intense, may for a time suppress discharges which are provoked by these stimuli when their intensity is weak. Quantitative data pertaining to those observations are presented.

5. Discharge characteristics of unitary responses to clicks are described and discussed.

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EFFECT OF POTASSIUM DEFICIENCY ON RENAL TUBULAR REABSORPTION AND ASSIMILATION OF GLUCOSE[1]

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It has been well documented that renal function during potassium deficiency is characterized by an inability to concentrate the urine (1), a decreased capacity to transport phenol red and *p*-aminohippurate (2), and an abnormality of urinary acidification consisting either of aciduria in the presence of extracellular metabolic alkalosis (3) or, in some instances, of an unusually alkaline urine due to the excretion of large amounts of ammonia (4). Within recent years isolated clinical reports have suggested that potassium deficiency may also be associated with a disturbance in glucose reabsorption. Stanbury and Macaulay (5) noted glycosuria in a nephrotic subject after the development of potassium depletion, and Earle *et al.* (6) has reported a patient with "potassium losing nephritis" whose maximal rate of glucose reabsorption was subnormal. It has also been postulated that the transport of glucose across cell membranes is related to the potassium ion. This has been particularly suggested by the simultaneous movement of glucose and potassium in association with glycogen accumulation or breakdown in the liver (7).

The present studies were undertaken to evaluate two separate aspects of glucose transport, first glucose reabsorption by the renal tubule, and second, the systemic assimilation, or uptake, of glucose.

METHODS

Trained unanesthetized female dogs were studied using standard clearance techniques. The maximal rate of glucose reabsorption (Tm_G) was determined during the hyperglycemia induced by the intravenous infusion of glucose with a constant speed Bowman infusion pump. Throughout the entire study, the dogs were placed on a constant diet. For dogs S and B, each day's ration consisted of one can of commercial dog food (425 gms.) to which was added granulated sucrose (75 gms. for dog B and 125 gms. for dog S) and 100 mEq. of NaCl. By analysis this diet contained 17 mEq. K, 132 mEq. Na, and 130 mEq. Cl. Dog M was maintained on a synthetic diet containing a similar amount of NaCl. The composition of this diet has been reported elsewhere (8); the daily

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[2] Holder of a Henry Strong Denison Scholarship.

intake of potassium was approximately 3 mEq. per day. After suitable control data had been obtained, potassium depletion was induced in all dogs by daily intramuscular injection of 25 milligrams of desoxycorticosterone acetate (DCA).[3] Maximal depletion was obtained in about two weeks, at which time the clearance experiments were repeated. Dogs S and B were then placed on a high potassium intake while the administration of DCA was continued. This consisted of an additional 67 mEq. per day of KCl added to the food; 0.15 per cent KCl was also substituted for the drinking water. On this regimen, the potassium deficit was corrected, and the effect of DCA itself was evaluated in the final set of experiments.

Starting with the beginning of the depletion period, the cumulative balances of potassium, sodium, and chloride were determined by the difference between food intake and urinary output. The animals ate all their food until the termination of the depletion period. However, the clearance studies were obtained while food intake was constant. Muscular weakness was observed in three dogs and one developed severe paralysis. Water intake and urine output increased two to three fold. Despite a constant food intake the animals lost approximately 10 per cent of their original weight.

For clearance experiments, the dogs were not anesthetized; urine was collected from an indwelling catheter. The exogenous creatinine clearance was used as a measure of the glomerular filtration rate. Glucose infusion (from 3 to 13 per cent) was administered at 15 ml. per minute. After 60 minutes equilibration, three or four consecutive clearance periods were obtained. Jugular venous blood was drawn at the mid-point of each period, immediately centrifuged, and the plasma separated from the cells. The arteriovenous difference of glucose was determined in several experiments and the maximal value was 7 mg. per hundred ml. Since all experiments were carried out at greatly elevated levels of blood glucose only a negligible error is introduced when venous concentrations, rather than arterial, are used for the present calculations.

CHEMICAL METHODS

Glucose was determined in duplicate by the Nelson modification of the Somogyi method (9); creatinine by the alkaline picrate method; sodium and potassium by internal standard flame photometry; chloride by the coulometric method of Cotlove *et al.* (10) and serum CO_2 by the manometric method of Van Slyke.

RESULTS

A typical protocol is shown in Table I. All the electrolyte balances and clearance experiments are summarized in Table II. The pattern of change in

[3] We are indebted to the Ciba Pharmaceutical Co. for generous supplies of Percorten Acetate.®

TABLE I

Protocols Showing Renal Tubular Reabsorption and Assimilation of Glucose During Control and Potassium Depletion Periods—Dog S

Day	Time	Urine Volume	GFR	Plasma Glucose	Glucose				
					Filtered	Excreted	Reabsorbed	Infused	Assimilated
Control									
	mins.	*ml./min.*		*mg. %*	*mg./min.*				
−22	−83	Priming infusion of 400 ml. of 6% glucose with creatinine.							
	−63	Infuse 6% glucose with creatinine, 15 ml./min.							
	0–24	12.0	88.4	384	340	66	274	900	834
	24–40	13.6	84.3	364	307	69	238	900	831
	40–53	12.5	87.2	382	335	99	236	900	801
Avg.			87	377			249		822
Potassium Depletion									
13	−79	Priming infusion 400 ml. of 3% glucose with creatinine.							
	−59	Infuse 3% glucose with creatinine, 16 ml./min.							
	0–12	9.2	79.3	511	406	144	262	480	336
	12–24	8.8	79.7	511	407	158	249	480	322
	24–36	9.9	79.2	502	397	168	229	480	312
	36–48	9.6	74.8	511	382	163	229	480	317
Avg.			78	509			242		323

serum electrolyte concentration corresponds well with that previously described for chronic potassium depletion, i.e. hypokalemia, hypochloremia and extracellular metabolic alkalosis. The serum sodium concentration showed no consistent change.

The cumulative balance data (Table II) demonstrate the production of a negative potassium and positive sodium balance during the depletion period, and also the production of a positive potassium balance during repletion. The degree of potassium depletion is to be considered as only an approximate estimate since fecal losses were not measured and nitrogen balance was not determined. Changes in body weight were slight but variable; none of the dogs developed diarrhea.

Measurement of the glomerular filtration rate and the glucose Tm showed essentially constant values during the control, depletion and repletion periods. The mean values of glucose reabsorption for all three dogs are—control: 189 ± S.E. 13.5; depletion: 179 ± S.E. 23.4, all values as mg./min. As indicated by the L/T ratios in Table II, the glucose loads were sufficiently great to saturate the reabsorptive capacity of the renal tubule. In addition, casual urines

TABLE II

Summary of Results on Electrolyte Balance and Glucose Metabolism

Day	Wt.	Cumulative Balance			Plasma					GFR	Tm_G	Glucose L/T Ratio	Glucose Assimilation
		Na	K	Cl	Na	K	CO_2	Cl	Glucose				
						Dog S							
					Control—Normal diet + 100 mEq. NaCl per day.								
	Kg.	mEq.			mEq./L.				mg. %	ml./min.	mg./min.		mg./kg./hr.
−56	21.4	—	—	—	156	4.0	23	120	1033	70	244	3.0	4,480
−50	21.4	—	—	—	147	3.8	18	117	1080	72	264	2.9	4,400
−32	19.6	—	—	—	142	3.4	22	105	953	74	235	3.0	4,910
−22	20.1	—	—	—	147	3.5	20	109	377	87	249	1.3	2,450
0		*Depletion*—Normal diet + 100 mEq. NaCl + 25 mg. DCA i.m. per day.											
13	17.8	+156	−248	−177	149	1.8	35	86	510	78	242	1.6	1,100
14	17.5	+173	−263	−172	—	1.7	—	—	622	75	260	1.8	1,055
15	16.8	+190	−278	−168									
16		*Repletion*—KCl added to diet and drinking water + 25 mg. DCA i.m. per day.											
30	17.9	+378	+61	+447	147	3.1	24	108	578	93	266	2.0	2,520
						Dog B							
					Control—Normal diet + 100 mEq. NaCl per day.								
−60	17.2	—	—	—	157	4.3	19	123	1050	48	129	3.9	4,500
−54	17.2	—	—	—	148	3.4	17	123	1360	50	131	5.2	4,000
−33	17.0	—	—	—	143	3.2	20	110	1025	41	108	3.9	4,150
−28	17.0	—	—	—	148	3.4	20	111	391	56	147	1.5	2,940
−20	17.4	—	—	—	149	3.6	19	108	454	53	147	1.6	2,900
0		*Depletion*—Normal diet + 100 mEq. NaCl + 25 mg. DCA i.m. per day.											
11	15.6	+118	−118	−39	154	2.1	32	98	1182	39	127	3.6	2,190
13	16.1	+182	−148	−9	154	1.8	—	102	388	56	137	1.6	1,500
15	15.6	+240	−166	+19									
16		*Repletion*—KCl added to diet and drinking water + 25 mg. DCA i.m. per day.											
31	16.5	+418	+124	+353	156	2.9	27	109	578	64	153	2.4	2,460
						Dog M							
					Control—Synthetic diet, 3 mEq. K + 100 mEq. NaCl per day.								
−42	17.3	—	—	—	155	5.7	—	108	610	67	213	1.9	2,890
−33	18.0	—	—	—	140	6.9	23	109	225	58	119	1.1	1,970
−26	17.2	—	—	—	—	4.9	21	115	388	74	231	1.3	2,990
−25	17.5	—	—	—	—	—	—	—	370	61	182	1.4	3,130
−15	17.9	—	—	—	—	—	—	—	624	63	198	2.0	3,690

TABLE II—*Continued*

Day	Wt.	Cumulative Balance			Plasma					GFR	Tm_G	Glucose L/T Ratio	Glucose Assimilation
		Na	K	Cl	Na	K	CO_2	Cl	Glucose				
0	*Depletion*—Same diet + 10 mg. DCA subcut. per day.												
	Kg.	mEq.			mEq./L.				mg. %	ml./min.	mg./min.		mg./kg./hr.
6	16.8	+519	−101	+544	152	1.8	28	104	940	57	150	3.6	3,710
21	15.6	+757	−365	+625	150	2.8	29	102	880	56	156	3.2	2,420

Plasma electrolyte levels were obtained before infusion of glucose. Plasma glucose represents average value during clearance experiment. Each glomerular filtration rate (GFR) and glucose Tm (Tm_G) are the average of 3 to 4 consecutive periods. In the next to the final column the glucose load (L) is calculated as the product of the plasma glucose concentration and the GFR; the load/transport ratio is denoted by L/T. Glucose assimilation is given in final column; for calculations see text.

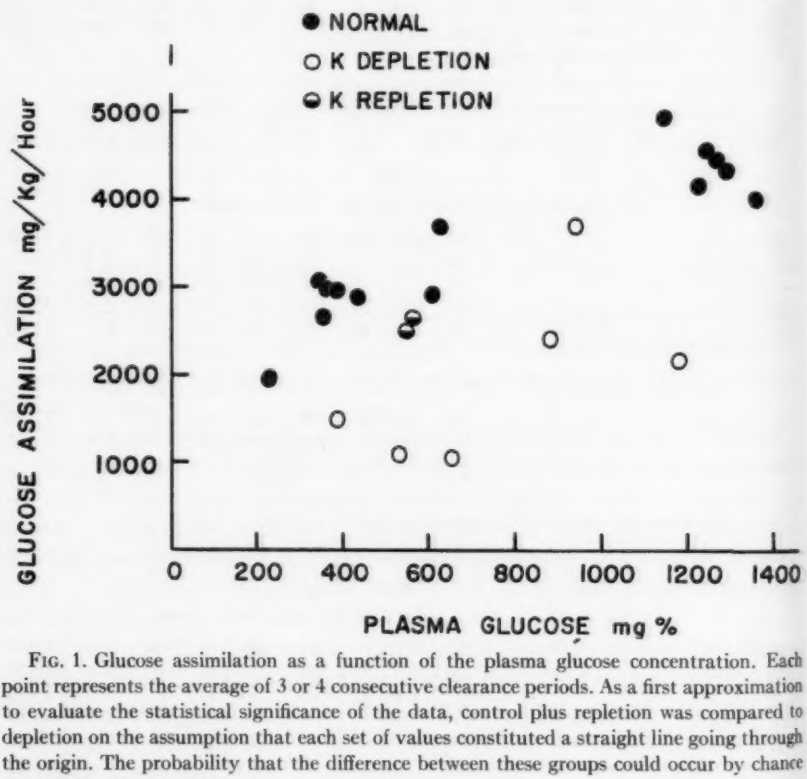

FIG. 1. Glucose assimilation as a function of the plasma glucose concentration. Each point represents the average of 3 or 4 consecutive clearance periods. As a first approximation to evaluate the statistical significance of the data, control plus repletion was compared to depletion on the assumption that each set of values constituted a straight line going through the origin. The probability that the difference between these groups could occur by chance alone is less than 1/1000.

were examined daily for glucose but no glycosuria was detected. The fasting blood sugar remained within normal limits.

For each experiment the rate of glucose assimilation was calculated as the difference between the amount of glucose infused and the amount excreted in the urine. Since the infusion rate was constant and the plasma levels of glucose showed only minor fluctuations during the brief period of observation, this calculation provides an accurate estimation of glucose retained within the body, but it is without implication as to the metabolic fate of the glucose. As indicated in Figure 1, the results confirm previous observations (see Soskin and Levine, 11) that glucose assimilation is dependent on the plasma glucose level. In potassium depletion the animals showed a marked decrease in their capacity to assimilate glucose. It may be noted that during depletion the highest (i.e. most normal) value in Figure 1 was obtained in dog M after only minimal potassium depletion of six days duration. When the potassium deficit was corrected, despite continued DCA administration, glucose assimilation returned towards control levels.

DISCUSSION

These studies have failed to demonstrate a defect in glucose reabsorption during potassium depletion in the dog, but have revealed a simultaneous abnormality in glucose assimilation. Many observations (see Relman and Schwartz, 12) have indicated that a fall in glomerular filtration rate is frequently associated with potassium depletion. However, filtration rate remained normal in these experiments as well as in others (8) performed with the same technique. It is clear that a decreased filtration rate is not a necessary sequel of a potassium deficit.

The normal rate of glucose assimilation is related to the plasma glucose level. Three possible mechanisms may be considered in explaining the "diabetic-like" behavior of the potassium-depleted animals. First, changes of this nature would be associated with starvation. Although the animals lost approximately 10 per cent of their weight over a two-week period, each dog had consumed all food up until the time the clearance studies were performed. Furthermore, potassium repletion was associated with a return of assimilation towards normal but without any significant increase in body weight. Therefore, it is unlikely that the findings are attributable to simple starvation. Second, a possible effect of the large amounts of DCA is ruled out by the return of glucose assimilation towards normal during the period of repletion. Third, potassium depletion itself might be directly responsible.

Several related findings have been reported by others. Gardner *et al.* (13) found that rats on a low potassium diet for 90 to 120 days showed a marked inability to metabolize a test dose of glucose, even though there was no hyper-

glycemia or glycosuria during the fasting state. They also observed that the glycogen content of the liver was markedly reduced. Glafkides (14) has reported that the increase in glycosuria following ACTH administration to diabetic rats could be prevented if potassium were administered concomitantly. In diabetic patients placed on ACTH, compound E or F, it has been reported that less resistance to insulin develops when KCl is administered (15). And finally, it has been reported that liver slices are unable to accumulate glycogen when incubated in a low potassium medium. This has been attributed to an increase in phosphorylase activity (16). These several findings, including our own observations, suggest that changes in potassium balance may have a direct effect on overall carbohydrate metabolism. It is emphasized that in these same animals, the potassium balance had no apparent effect on glucose transport by the kidney. Parenthetically, it may be noted that a similar phenomenon is observed with insulin. This agent has a marked effect on cellular permeability to glucose (17), but it does not increase the tubular transport of glucose (18).

SUMMARY

Potassium depletion was obtained in the dog by a relatively low potassium intake and by the administration of DCA. Under these circumstances a significant potassium deficit was not accompanied by any change in the reabsorption of glucose by the kidney. However, glucose assimilation was impaired. Considerable ancillary evidence suggests that this latter effect may be a direct consequence of potassium depletion.

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THE ISOLATED CIRCULAR INTESTINAL LOOP

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The purpose of this communication is to report the surgical construction, in dogs, of isolated, circular, intestinal loops. The authors believe that this experimental preparation, hitherto undescribed, may prove to be useful in studying the secretions of the various components of the gastrointestinal tract, as well as the absorption and excretion of various substances.

In brief, whereas previous experimental preparations involving isolated intestinal segments employed either closed loops (with blind ends) or external fistulae, in this experiment the ends of the isolated segment were anastomosed so that a true circular loop was formed. The isolated circular loop may be composed of any portion or portions of the small and large intestine, depending upon the objectives to be studied. Furthermore, a part of the loop may be placed in a subcutaneous position for easy access by needle (and fine polythene tube) to the lumen. A functioning gastrointestinal tract was then reconstituted by anastomosing those portions of the tract which were not included in the isolated loop. Several end-to-end anastomoses were needed for the construction of the more complicated preparations.

Isolated circular intestinal loops have been constructed in 33 mongrel dogs. Long-term survivals, upwards of 60 days, have been obtained when the isolated loop contained neomycin and included either ileum or colon. Under these conditions, the isolated circular loop did not become dilated, and the mucosa was not ulcerated. There was considerable variety with regard to components of the intestinal segments and their lengths. The authors have constructed only a few types, with the following results:

1. Isolated circular loop composed of the entire duodenum and a short length of contiguous jejunum. No animal survived beyond 8 days. Uniformly the loop was dilated, ulcerated, and perforation occurred.
2. Isolated circular loop composed of jejunum and ileum. Long-term survival has been observed. Autopsy at 50 days following operation showed moderate dilatation of the loop but no ulceration of mucosa.
3. Isolated circular loop composed of ileum and contiguous colon. Long-term survival has been observed, upwards of 60 days. In these preparations the loop, at autopsy, was not dilated and not ulcerated.
4. Isolated circular loop composed of duodenum, ileum and contiguous colon. These animals succumbed in 4 to 6 weeks from jejunal ulceration at the

gastrojejunal suture line. At autopsy there was no dilatation of the isolated loop and no ulceration of its mucosa.

5. Isolated circular loops composed of either jejunum or ileum but without neomycin in the loop. No animal survived beyond 24 days. Of chief interest is the fact that these animals all lived longer than would have been expected had the isolated loop been blind rather than circular.

Additional variations are currently being constructed. It seems clear, however, that the circular arrangement of the isolated loop will permit metabolic and other studies of a reasonable duration to be made without the complicating features of fistula or infection. At the same time, the animal retains the ability to eat and thus maintains a normal metabolic and fluid balance.

SUMMARY

Isolated circular intestinal loops have been constructed in 33 dogs. When these loops contain neomycin and include ileum or colon, long-term survival has been observed. The authors believe that this new experimental preparation will be useful in studying intestinal absorption and excretion.

ABSTRACTS OF PAPERS PRESENTED BEFORE THE MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

February 9, 1959

THE PROTEIN BINDING OF BILIRUBIN AND ITS RELATIONSHIP TO KERNICTERUS

GERARD B. ODELL

The protein binding of bilirubin was studied in the serum from jaundiced newborn infants and in a model serum prepared from bovine albumin and crystalline bilirubin made up in an isotonic phosphate buffer of pH 7.4.

Certain organic anions used in clinical medicine were found to uncouple bilirubin from albumin in both human and model serum. The organic anions tested were the sulfonamides, sulfisoxazole, sulfadiazine and sulfamethoxypyrimidine and salicylate. The concentrations of organic anion employed were in amounts comparable to those used in clinical medicine. The amount of uncoupling of bilirubin was estimated by a spectrophotometric technique and by dialysis. The amount of uncoupling of protein-bound bilirubin was found to be proportional to the relative saturation of the albumin with bilirubin and to the amount of organic anion present. The relative ability of the organic anions in decreasing order of effectiveness to uncouple protein bound bilirubin was salicylate > sulfamethoxypyrimidine > sulfisoxazole > sulfadiazine. The results of the study indicated that the clinical use of these drugs in hyperbilirubinemic newborns would enhance the development of kernicterus by increasing the diffusion of bilrubin into brain tissues. The increased diffusion would occur because these drugs would displace bilirubin attached to plasma proteins and thereby increase the diffusible bilirubin.

HEPATIC COMA—A DEPLETION AND REPLETION SYNDROME

ALICE N. BESSMAN AND GEORGE S. MIRICK

It is generally agreed that elevated blood ammonia levels play an important role in the development of hepatic coma. However the lack of correlation between these levels and the state of consciousness of the patient in a certain number of cases has raised doubt as to the true significance of blood ammonia in producing hepatic coma. We have studied twelve patients going in to or coming out of hepatic coma. Serial ammonia levels (arterial and venous) were measured as the patient became more or less obtunded. In all of the cases studied, the degree of coma increased as the ammonia levels rose, or the degree of coma decreased as the ammonia levels fell. In five of the patients there was a good immediate correlation between the blood ammonia levels and the degree of coma. In seven patients there was a lag of from twelve to seventy-two hours between the change in blood ammonia levels and the change in the sensorium of the patient. Two patients demonstrated elevated ammonia levels twelve to twenty-four hours prior to becoming obtunded. In five other patients the ammonia levels were lowered to normal or near normal levels but clinical improvement lagged behind biochemical improvement by twelve to seventy-two hours. We feel that these findings are consistent with the theory that ammonia produces coma by interference with the utilization of glucose by the brain. Specifically the lesion is one of depletion of Krebs cycle intermediates by reversal of the glutamic dehydrogenase reaction in the presence of elevated ammonia levels. However this reaction and the subsequent depletion of the intermediates is dependent not only on the degree of ammonia elevation but also on the duration

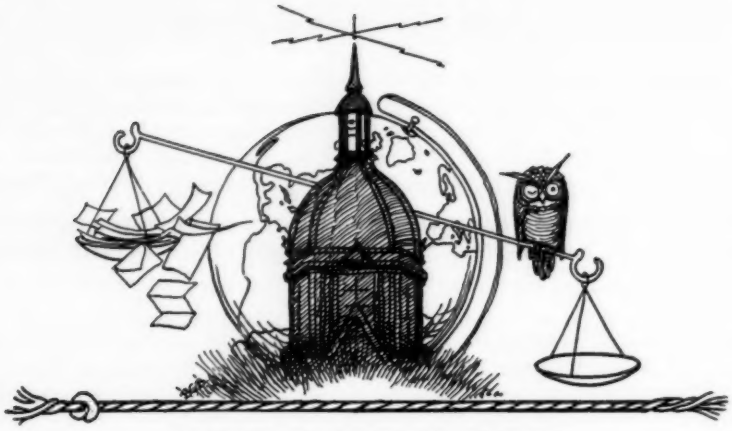
that this elevation has been present. Likewise the depletion and subsequent repletion of the cycle components, and recovery of mental function are probably influenced by other factors such as anemia, uremia, etc. In view of this, although an immediate blood ammonia level may correlate well with the clinical picture in most instances, in some situations, *only serial* determinations will reveal the correlation between the clinical and the biochemical findings.

CELLULAR CHANGES IN VAGINAL AND BUCCAL SMEARS AFTER RADIATION: AN INDEX OF THE RADIOCURABILITY OF CARCINOMA OF THE CERVIX

HOWARD W. JONES, JR., BENJAMIN GOLDBERG, HUGH DAVIS AND BEAURY C. BURNS, JR.

Following the technique of Graham, changes in the normal exfoliated vaginal cells after radiation have been tabulated. These alterations were correlated with the clinical results at the end of two years in approximately 60 patients. No difference in end results was noted in those patients with a good radiation response as compared with those with a poor radiation response.

An attempt was made to test the generalized post reaction to radiation by following the changes in the cells of the buccal mucosa after a standard test dose of radiation. With this material with a somewhat fewer number of patients there was a suggestion that patients with a high radiation response had a better end result than patients with a low radiation response. The total desoxyribose content of the nuclei of radiated cells was measured. The possible significance of these changes to the end results was discussed.



BOOK REVIEWS

Dynamics of Proliferating Tissues. Edited by DOROTHY PRICE. A Report from The Developmental Biology Conference Series, 1956. 96 pp., $3.25. The University of Chicago Press, Chicago, Ill.

The Developmental Biology Conference Series was held in 1956 under the auspices of the National Academy of Sciences-National Research Council. It was organized to foster a critical, multidisciplinary consideration of our knowledge of the spectrum of processes which constitute the biologic phenomena of growth and development. A record of these conferences is being published in ten small volumes, each prepared by an experienced scientist who attended the sessions.

"Dynamics of Proliferating Tissues" is one of these ten reports in which Dorothy Price has abstracted the presentations and comments of such conferees as Bessis, Bierman, Congdon, Jacoby, Matoltsy, Osgood, Teir, and Weiss. These scientists reviewed the current methodology used in studying cellular proliferation and loss and the physical and chemical environmental factors which affect these processes. Particular attention is directed toward the examination of these phenomena in the skin and hematopoietic systems.

The entire conference is reported in a brief third person account which deprived this reviewer of the sense of "being present" which is engendered by many more extensive dialogue style accounts of similar symposia. This publication, however, has preserved the identity of each contributor, abstracted his presentations and included a bibliography containing items dated through 1957. It thus serves to effectively introduce each reader to several approaches to these biologic processes and to more clearly define certain problems currently under scrutiny.

ALBERT H. OWENS, JR.

Fracture Surgery. A Textbook of Common Fractures. By HENRY MILCH AND ROBERT AUSTIN MILCH. 470 pp., $17.50. Paul B. Hoeber, Inc., New York, N. Y.

In recent times textbooks on fractures have been divided into very large and thorough works and rather simple handbooks. The authors of *Fracture Surgery* have sought to bridge the gap with a book of intermediate scope intended for medical students and surgeons of limited fracture experience. They have fulfilled this intermediate position very well, but like all such works, they may be easily criticized for their selection of topics to be emphasized. The first section and one-third of the book is concerned with general aspects of fracture

treatment and gives a brief review of the general treatment of surgical trauma. There is an excellent chapter analyzing the displacement of fracture fragments which will be of great aid to the novice in interpreting his x-rays and in analyzing his manipulations. The usual types of traction and some of the commoner internal fixation devices are adequately described. The remaining two-thirds of the book are concerned with the diagnosis and treatment of most of the commoner fractures and these are in adequately detailed and portioned sections.

The assets and debits of this book are best summarized by comparing it to the major fracture works with which it must compete. It does not give the excellent description of the manipulation of closed fractures found in Charnley, but it does give much instruction in this matter. It is not as pleasant reading as Watson-Jones, but it is not so long and very practical. It does not give the detail and alternative methods of Key and Conwell, but it concisely describes an accepted way of handling each of the fractures described. Operative methods are described in sufficient detail to inform the medical student or physician who will not be doing such surgery but I doubt if any surgeon will find the book adequate for technical information preliminary to such surgery. The format, organization, and illustration of the book are good.

We recommend this book as a basic or first book in the library of students and doctors who wish to learn fractures. It is an excellent step past many of the extremely concise handbook texts now available and which are often so quickly outgrown in experience.

GEORGE W. SETTLE

Ciba Foundation Symposium on Amino Acids and Peptides with Antimetabolic Activity. Edited by G. E. W. WOLSTENHOLME AND CECILIA M. O'CONNOR. 286 pp., $8.75. Little, Brown & Co., Boston, Mass.

The chemical and biologic properties of several amino acids and peptide derivatives which possessed antimetabolic or cytotoxic properties was the subject of a conference sponsored by the Ciba Foundation in March 1958. Twenty-nine scientists participated including Bergel, Buchanan, Craig, Farber, Gale, Sheehan, Skipper, and Stock, and this record of papers and discussions is set forth in the familiar style of the Ciba Symposia.

The antitumor and antibacterial effects of several compounds were considered. Such agents as chloramphenicol, etamycin, bacitracin, actinomycin, azaserine, and amino acids carrying nitrogen mustard groups are considered with regard to their chemical synthesis, their relationship to purine and protein biosynthesis, their possible sites and mechanism of action, and the structure-activity relationships which have suggested themselves to date. The critique of current achievements and the many speculations made by these well qualified investigators are preserved in the discussions. These passages are of particular interest in view of the extent of our current knowledge of this area and its rapidly developing technology.

The reader is also provided the opportunity of appreciating the limited clinical achievements thus far made possible by the more basic biochemical investigations. To this end a few clinical studies of the antitumor action of the actinomycins and amino acids carrying nitrogen mustard groups are briefly summarized. In general, the main value of this volume lies in its multidisciplinary consideration of this group of compounds.

ALBERT H. OWENS, JR.

A Symposium on the Chemical Basis of Development. Edited by WILLIAM D. MCELROY AND BENTLEY GLASS. 934 pp., $15.00. The Johns Hopkins Press, Baltimore, Md.

Ross Harrison, whose words will be valued at their true worth by readers of this Bulletin,

once said about twenty-five years ago that summing up embryology was like summing up a dictionary. Embryology at that time was at a peak of popularity. Its phylogenetic phase, so heavily exploited at the end of the nineteenth century, had by then become of no more than historical interest; while students in medical schools were studying the morphology of embryos, laboratory investigators were applying new and exicting experimental techniques, in great part as a result of the work of Harrison himself, to the causal analysis of the sequence of events in time which the embryo carries off with so much finish. No completely adequate summary of this phase of embryology has ever been made available. Spemann covered a portion of it in his Silliman Lectures in the 1930's; Harrison's own Silliman Lectures, delivered in the 1940's and now being prepared for publication, help to complete the story.

But during the 1930's embryology lost its high favor, partly because work in other fields, particularly in biochemistry and genetics, promised suddenly to become more animated, partly because embryology itself, in adapting to its new intellectual environment, asked the wrong questions. What is *the* substance, it began by asking the biochemists, that is *the* organizer of embryonic development? Since there can be no right answer to the wrong question, the enthusiasms waned that had been generated by the discovery of the organizer, and embryology had to await new developments before its ascendency was restored.

Embryology, now considered by some to be broadened by the expedient of considering it part of a wider group of studies called developmental biology, has however finally again been returned to popularity, this time not as the result of any dramatic new discovery, but by its rapprochement to biochemistry and genetics, and perhaps also immunology, of which it is now asking questions far more limited than before, but questions that are at least beginning to be answered in a limited vocabulary. These are the questions that were discussed in March 1958 in the McCollum-Pratt Symposium on the Chemical Basis of Development.

The Symposium was planned to honor Professor Benjamin H. Willier, the retiring Chairman of the Department of Biology in The Johns Hopkins University, whose excellence of teaching and of investigation has so greatly enhanced progress in developmental biology. The volume attains the usual high standards of the McCollum-Pratt Symposium volumes. The published papers are grouped around four central topics: developmental cytology, cellular and tissue interactions in development, problems of specificity in growth and development, and control mechanisms in development. Medical men who remember the study of development as interpreted in terms of cross-sections will through reading this book come to understand both the scope and the variety of the new problems investigated by developmental biology. Medical thinkers who are themselves concerned—and who among them are not?—with problems relating to biochemistry and genetics will learn from it the importance of the central position which developmental biology now holds in relating these fields to each other.

Forty-two papers are included in the volume, and a number of other contributions reported in the discussions. Like Harrison's dictionary, they defy summing up. But both Paul Weiss in some final remarks at the end of the symposium, and Bentley Glass in his excellent epitome, give expression to what was felt throughout the Symposium, that the fundamental problem of development—the relationship between structure and chemistry in organization—remains largely untouched. Yet there was also an excited anticipatory sense throughout that developmental biology is on the threshold of great advances. Our crossing it is being prepared for by the contributors to this volume, the reading of which will therefore amply reward the readers of this Bulletin.

JANE OPPENHEIMER

Polarography in Medicine, Biochemistry and Pharmacy, Revised English Edition. By M. BREZINA AND P. ZUMAN. Translated by S. WAWZONEK. 862 pp., $19.50. Interscience Publishers, Inc., New York, N. Y.

The original Czech edition of this text was designed to familiarize biologists with the potential of polarographic analysis and to provide a convenient comprehensive summary of the world literature on the biological applications of polarography. This revised English version can serve both purposes for American biologists, especially since a good deal of the literature originally appeared in Slavic languages which usually present difficulties in translation. The book consists largely of fairly detailed descriptions for the preparation of biological samples for polarographic analysis of both organic and inorganic substances. However, no detailed introduction to polarographic technique, instrumentation or theory is included. The authors usually include the biological interpretation of results obtained with polarographic techniques; this is of little value. The usefulness of the volume would be considerably enhanced had it been stated for each procedure whether or not the original paper contained data on the accuracy and specificity of the method. The numerous illustrations of polarograms, tables of half-wave potentials and two indices (substance index and subject index) contribute greatly to the usefulness of the book. The section on enzymes illustrates the usefulness of polarography in studying reaction rates and should stimulate more widespread consideration of this technique. It is anticipated that this volume will be of more use to research and pharmaceutical institutions than to routine clinical laboratories.

I. M. WEINER

Chemical Prevention of Cardiac Necroses. By HANS SELYE. 235 pp., $7.50. The Ronald Press Co., New York, N. Y.

Dr. Selye summarizes in this monograph a great quantity of literature concerning the experimental production of myocardial disease by diverse agents (hypersensitivity, infections, hypoxia, chemical and physical agents, dietary factors, psychic and physical stress) and the results of his own investigations in experimental cardiopathy, much of which has also been published previously. Particular emphasis is placed on the production of myocardial lesions by administration of corticosteroids (of which 2α-methyl-9α-chlorocortisol was most effective) and various electrolyte solutions (especially Na^+, $PO_4^{\equiv}$, $SO_4^{=}$, and ClO^-) to laboratory animals; the ability to prevent these effects by the administration of certain other substances (especially K^+, Mg^{++}, and Cl^-); and the augmentation or inhibition of the effects of various unrelated noxious agents; many of which are associated with myocardial disease in man, by steroid and electrolyte "conditioning."

The author draws attention to the nonspecific nature of the pathological changes of inflammation and necrosis observed in human and experimental heart disease and suggest that "stress" constitutes the common etiological factor. "Presumably, there exists in the heart a particularly labile chemical system, which is easily deranged through some metabolic effect of stress, especially in the presence of intense hypercorticoidism and an excess of sensitizing sodium salts." As he admits, the large body of fragmentary evidence gathered along these lines thus far serves chiefly to indicate a much greater amount of work that remains to be done before the hope is justified that these investigations might lead to the understanding, prevention, and therapy of cardiac diseases in man.

PATRICIA M. SMITH

Treatment in Internal Medicine. By HAROLD THOMAS HYMAN. 609 pp., $12.50. J. B. LIPPINCOTT Co., Philadelphia, Pa.

This volume was planned as an "office and bedside consultant to colleagues who deal

first-hand with the problems of daily practice." As the reviewer first scanned its contents he wondered if another such manual were really necessary. However, as his exploration of the text proceeded, several features proved to be pleasant surprises.

It is no mean feat for one author to consider such a broad topic as "Treatment in Internal Medicine" in light of the many recent therapeutic and pharmaceutical advances. Dr. Hyman has done so quite authoritatively. His material is set forth concisely and in a well indexed and highly organized manner. His recommendations are leavened with the common sense of a mature practitioner. The merits of therapeutic alternatives are discussed briefly and reference is made to the recent pertinent literature. The problems involved in treating a wide variety of common complaints are considered in some detail and specific instructions for the completion of various common and therapeutic procedures are also presented.

Physicians should find "Treatment in Internal Medicine" a ready and reliable "consultant" indeed.

ALBERT H. OWENS, JR.

The Integration of Behavior. Vol. III, The Reintegrative Process in a Psychoanalytic Treatment. By THOMAS M. FRENCH. 484 pp., $10.00. The University of Chicago Press, Chicago, Ill.

This is a psychoanalytic study of human integrative functions. The author scrutinizes certain ego processes in his third volume. He uses detailed hour to hour accounts of two years of analytic therapy of a neurosis ridden man afflicted with bronchial asthma. This patient exemplifies what Adolph Meyer has termed the human personality as "an experiment of nature" via the experimental medium of Freud's analytic relationship. The content of the patient's conflicts, hopes, fears and changes is mainly derived from dreams.

The author defines the ego, or integrative mechanism, of the personality as the problem solving apparatus. In contrast to most other investigators of recent years (for example, Anna Freud's, The Ego and Mechanisms of Defense) in which the ego is emphasized in its defensive role as a sort of fire department, Dr. French holds that it has mainly a need-satisfying, problem-solving function. He stresses the opinion that neurotic illness is the result of interruption of healthy learning process by emotional traumata, either acute or continual as a more or less chronic interference to growth. The aim of psychoanalytic therapy is to restore the learning process by bringing about the fullest integrative capacity. The latter can bring to bear its constructive force in problem-solving once the patient in a suitable relationship with the doctor has been able to appreciate his historically determined conflict (mobilized in therapy) and has safely re-experienced it in the light of present reality. His integrative functions then have a more or less fair chance of bringing him to the point of discarding anachronistic hopes and responses. In relative freedom from presently inappropriate modes of feeling and behavior he can learn, grow and function less hampered by the burdensome aspects of the past. He no longer must commute to work via horse and buggy in an age of high compression locomotion.

The integrative capacity is limited, according to Dr. French, by (1) a factor known as constitution, an inherent quantum, (2) by the effects of stress,—external or internal and (3) by the amount of hope (that is, ability to tolerate pain and frustration—otherwise a type of long range optimistic determination). The integrative task is increased by stress, especially the stress of a mobilized conflict which is of necessity a part of successful analytic therapy.

This interesting study is technical but it is valuable stimulation to any student of human behavior, especially to those in the field of medical psychology.

WILLIAM T. DIXON

Difficult Diagnosis: A Guide to the Interpretation of Obscure Illness. By H. J. ROBERTS. 913 pp., $19.00. W. B. Saunders Co., Philadelphia, Pa.

In this book Dr. Roberts has attempted to collect a large number of the less well-known facts about both common and unusual diseases. Instead of taking the conventional approach to disease followed in standard text-books of medicine, the author has divided the text into several broad categories such as endocrinopathies, metabolic disorders, fever and infections of obscure origin, neoplastic diseases, dyscollagenoses, etc. Under each of these groupings he discusses briefly some of the more interesting and frequently unappreciated facets of various diseases. For example, he advises that pyelonephritis be seriously considered in the following clinical situations: (1) rapidly progressive hypertensive vascular disease in young patients with a negative family history (2) excessive anemia in patients with malignant hypertension (3) toxemia of pregnancy (4) in patients with unexplained spinal osteomyelitis and (5) in unexplained chronic renal disease characterized by marked fluctuations in renal function. In other words, the author has assembled a valuable collection of 'clinical pearls' which should enhance the knowledge of every internist.

The greatest value of this book, however, lies in a superb bibliography. The author has culled from the literature close to 3000 references, and most good clinical contributions which have appeared in the English literature during the past decade have been included. If one were to compose a reading list for medical students, one could do little better than to recommend this bibliography *in toto*.

Other unusual features of this book include a chapter on iatrogenic disease which emphasizes adverse reactions seen with many of the new therapeutic agents. There is an atlas featuring 100 illustrations of common dermatologic disorders, and a section on the value of certain useful diagnostic procedures. Finally, the book is carefully indexed according to signs, symptoms and laboratory manifestations of disease.

It should be pointed out that this book is not written for beginners, and the author presupposes a solid grounding in medicine. On the other hand, for the sophisticated clinician many sections are delightful. For those interested in clinical 'ploys' and advanced 'CPC-manship', this is probably the text of choice. Aside from these academic considerations, however, clues gleaned from perusal of this book will lead to the correct solutions of some difficult diagnostic problems. This book is a valuable contribution and it is recommended tha all practicing internists add it to their list of reference books in medicine.

ROBERT G. PETERSDORF

Bacterial and Mycotic Infections of Man, 3rd edition. Edited by RENE J. DUBOS. 820 pp. $8.50. J. B. Lippincott Co., Philadelphia, Pa.

The first two editions of Dubos's textbook of microbiology have been widely used and acclaimed. There is good reason to suspect that the third edition will be similarly received.

The book has been largely rewritten and two new chapters, "The Evolution and the Ecology of Microbial Diseases" and "Chemotherapy of Microbial Diseases" have been added. In the first of the new chapters, Dr. Dubos speculates on the answers to the question "Why do pathogens so often fail to cause disease after they have become established in the tissues?" The carrier state, latent infection, and properties of bacteria and host that are responsible for disease make up the other part of this chapter. In discussing antimicrobial therapy Dr. Walsh McDermott deals with two relatively neglected aspects of antibiotic therapy—drug-host, and drug-parasite interaction.

The sections on immunology are not greatly expanded and do not emphasize quantitative immunochemistry. Curiously, there is no systematic treatment of the non-hemolytic *strep-*

tococci. The strength of this book lies in the emphasis on pathogenesis of infectious disease and the correlation of microbiology with clinical medicine.

JOHN C. RIBBLE

The Care of the Geriatric Patient. Edited by E. V. COWDRY. 438 pp., $8.00. The C. V. Mosby Co., St. Louis, Mo.

The over-all effect created by the book is one of optimism. Statistically and actually, geriatrics is a branch of medicine which is literally in its infancy, having only been recognized as a science for the past two or three decades. And yet, as each of the contributors graphically points out, the specific effects of aging upon each of an individual's organ systems and upon his personality structure, (including cerebration and mentation) are indeed quite measurable in many instances, and of critical clinical significance at all times.

Chapter two, "Psychologic Aspects in the Management of the Geriatric Patient" by Wilma Donahue, is particularly revealing and in general points out that individuals age psychologically as well as physiologically and medically. The author discusses these psychological changes under four categories: intellectual, psychomotor, perceptive, and personality. It is strongly recommended that any and all physicians who treat geriatric patients be cognizant of the psychologic stresses and changes of aging, in order to enable the elderly patient to make maximum use of his adaptive efforts, intrapsychically and environmentally.

Chapter sixteen, "Rehabilitation of the Geriatric Patient" by Sedgwich Mead, is also extremely useful in that it summarizes and attempts to integrate most of the medical, surgical, endocrinological and psychiatric aspects of aging in a meaningful way. When this is accomplished, the elderly patient is able to continue functioning efficiently on as high a level as possible, physically, medically, socially, and psychologically. The author points out that these goals can most frequently be reached by an understanding and capable physician utilizing the comprehensive approach. The author wisely points out that whereas maximum rehabilitation for one patient may enable him to return to competitive employment, for many others, it would enable him to go from a chronic hospital to home care, or perhaps to become ambulatory after being bed fast.

The last chapter, "Geriatrics Around the World" by J. H. Sheldon, graphically illustrates the depth and scope of the multiple problems of aging on an international as well as on a local and national level. Many European countries have advanced programs dealing with many of the specific medical and socioeconomic problems of aging.

It is significant that when the International Association of Gerontology was found in 1950, there were twelve countries as foundation members, and in 1954, forty-two countries were represented. International Congresses of the organization are held at triennial intervals, the last being held at Merano, Italy in July, 1957.

Interchange of information on all levels dealing with all aspects of geriatrics, is believed to be essential in solving the multidisciplinary problems of aging, which almost every country is experiencing as the number of persons over sixty-five years of age progressively increases at an alarming rate. The world-wide peak will not be reached for at least several decades, but even now, the percentage of persons over sixty-five in most countries has doubled or tripled in the last twenty-five years.

This means that all physicians will be seeing more and more older patients unless they restrict their practices. The book is therefore recommended to all physicians. Those who read it will profit by reading specific chapters or even more by reading the entire 416 pages.

It was also wisely recommended by various contributors that only physicians who actually *like* older patients should specialize in geriatrics. Astute clinicians generally can attest to the wisdom of this philosophy, from the doctor's as well as from the patient's point of view.

The value of this book is apt to increase in the years to come, statistically and quantitatively, in terms of the number of patients who are reaching the older age groups, and qualitatively in terms of the specific information presented by the various specialists, which has definite clinical utility.

JULIAN REED

Metabolism of Lipids. British Medical Bulletin, Vol. 14, No. 3, Sept. 1958. 278 pp., $3.25. Published by the Medical Department, The British Council, London, England.

This monograph contains twelve presentations by twenty British investigators in this important field from the points of view of anatomy, biochemistry, cardiology, endocrinology, nutrition, pathology, and radiopathology. The papers "touch on most of the major issues that are in the forefront of advancing knowledge at the present time."

Specific papers deal comprehensively and critically with such vital subjects as the biosynthesis of sterols, lipid absorption and transport, hormonal effects upon lipogenesis and control of circulating lipids, dietary fats and ischaemic heart disease, lipids and blood coagulation and finally the relation of essential fatty acids to human disease.

The monograph is elegantly illustrated and the references are broad in scope.

For the clinician there is ready enlightenment upon basic concepts and for the preclinical investigator a sound orientation in clinical relationships. Throughout the monograph there is recurring stimulation to explorers to concern themselves with wide areas of deficiency in our knowledge.

BENJAMIN M. BAKER

Chronic Illness in the United States, Vol. IV. Chronic Illness in a Large City. By Commission on Chronic Illness. 620 pp., $8.00. Published for the Commonwealth Fund by Harvard University Press, Cambridge, Mass.

This book is the fourth and final volume of a recent report of the Commission on Chronic Illness. In 1950 the American Hospital Association, American Medical Association, American Public Health Association, and American Public Welfare Association established the Commission as an independent, voluntary organization. The purpose of the program was to assess the magnitude of the chronic illness problem with the hope of laying the foundation for the prevention and control of these diseases. Volume I considered *Prevention of Chronic Illness*; Volume II, *Care of the Long Term Patient*; and Volume III, *Chronic Illness in a Rural Area*.

Volume IV presents the results of their studies of chronic illness in Baltimore, selected as a typical urban area. The goal was to obtain basic facts about the chronically ill—who they are, how many there are, their ages, the kinds and extent of their disabilities, their characteristics, and the care they need.

In the initial phase of the study, approximately 4000 Baltimore households were selected by the Bureau of Census as being representative of the entire population. One out of 80 addresses was selected at random. Medical institutions for long-term care—that is, chronic disease hospitals, nursing homes, and homes for the aged—were not included. Seven interviewers trained by the Bureau of Census surveyed 11,574 persons. One out of 7 persons (144 per 1000) in the sample interviewed was bothered by some illness on the day preceding the interview; 29 per 1000 persons were disabled; 15 per 1000 were in bed as a result of illness. Eleven per cent had been disabled one or more days during the four weeks preceding the interview.

The second phase was a clinical evaluation of 722 persons by physicians at a special clinic established for the purpose at the Johns Hopkins Hospital. Forty-five of 385 persons

who refused or were unable to come to the clinic were evaluated on the basis of existing hospital medical records. Forty-two additional persons were examined in the home.

Nearly 1600 chronic diseases per 1000 population were diagnosed in the 809 persons evaluated. Prevalence rates for each chronic disease were determined. For example, the prevalence of heart disease of all types was found to be 96 per 1000 population. The rate for hypertension without heart involvement was 66 per 1000. Arthritis with a prevalence of 75 per 1000 persons was exceeded by the heart disease rate and the rate for mental disorders (109 per 1000). One-eighth of the diseases were judged to be severe; another 25 per cent were moderate in severity. Fifty-six per cent were judged to be "substantial," that is, they interfered with or limited the person's activities, required care or were likely to do either of these in the future. Fifty-six per cent of the *people* had at least one substantial disease.

An evaluation was made of the extent of the disability resulting from chronic illness. Three per cent of the persons interviewed believed they were disabled, that is, kept from their usual activities. According to the evaluation of the examining physicians, 4 per cent had limitation of normal activity and 6 per cent had limited ability to work.

The third phase of the study was an evaluation of the usefulness of screening procedures as a means of obtaining prevalence data. Approximately 7000 persons were contacted by means of several letters, telephone calls, and personal calls to the home, together with the encouragement provided by one radio and four television programs. Only 25 per cent (2024 persons) responded. Screening procedures consisted of determination of height, weight, blood pressure, urine and blood sugar, hematocrit, urine albumin, and serological tests for syphilis. Audiometry, visual acuity testing, and chest x-ray were also done. Thirty-two per cent of those screened were found to have one or more "major" abnormalities. As a result of the screening, 607 persons were advised to go to their personal physicians. 351 (58%) actually reported to the physicians for examination.

The final phase was an attempt at rehabilitation for those persons identified by clinical examination as having a handicap believed to be amenable to rehabilitation. Using as criteria the policies and regulations of the Maryland vocational rehabilitation program, an evaluation team estimated that in 36 cases a potential for rehabilitation existed. An attempt was made to rehabilitate these persons. In only four persons was the rehabilitation completed. One man was gotten a job as a night-watchman; a herniorrhaphy was arranged for another man who subsequently located a job himself; one woman had correction of a cystocele and rectocele and then went to live with her son; and a fourth man was placed in a job as a night-watchman. Assuming that all cases still in the process of rehabilitation were eventually rehabilitated, the rehabilitation rate is still only 1.8 per 1000 population of the total group evaluated.

440 persons of 809 clinically evaluated were considered a high disability group, i.e. they had maximal disability, diabetes mellitus, central nervous system disease, neoplasms, heart disease, arthritis or rheumatism. Ninety per cent of these persons were believed to need general medical supervision and care. Thirty-eight per cent needed aids in financing medical care. Thirty-two per cent needed specialist consultation. Twenty-seven per cent needed nursing care. Only 1 per cent needed institutional care, 2 per cent needed occupational therapy, 7 per cent needed self-help devices, and 13 per cent needed psychiatric care. In view of a tendency to believe that psychiatrists, social workers, occupational therapists, and vocational rehabilitation counselors are the answer to the chronic illness problem, the fact that 90 per cent were thought to need general medical care is of great importance.

Another important finding was that the household interview by trained lay personnel was not a sensitive or accurate method of determining the prevalence of chronic illness. Two to three times as many conditions were found on clinical evaluation by physicians. Further-

more, when the results of examinations by physicians were compared with the results of the household interview, diagnoses matched in only 22 per cent of the diseases.

People's attitudes toward health and medical care were found to be major obstacles to the application of available therapeutic measures. Of the selected high disability sample, more than 50 per cent were judged to be neglecting their disease. More than one-third ignored a recommendation to obtain medical care. Furthermore, people in general were reluctant to accept the diagnostic study offered them in the clinical evaluation.

At its conception, the study was unique in its aims to get data on the chronic illness problem. Subsequently, several other organizations, such as the Health Insurance Plan of Greater New York, have also attempted to study the problem of chronic illness. The study in Baltimore should be required reading for politicians, psychiatrists, social workers, vocational rehabilitation counselors, public health physicians and other physicians who have a broad outlook on medicine. In addition to being a classic in the field of public health, it is well written and makes fascinating reading.

HENRY N. WAGNER, JR. AND ALFONSE T. MASI

Cold Injury, Ground Type. By COL. TOM F. WHAYNE AND MICHAEL E. DEBAKEY. Prepared under the direction of MAJ. GEN. S. B. HAYS. Editor in chief, COL. JOHN BOYD COATES, JR. Associate editor, ELIZABETH M. MCFETRIDGE. Office of the Surgeon General, Dept. of the Army, Washington, D. C. 570 pp., $6.25. Superintendent of Documents, U. S. Government Printing Office, Washington, D. C.

Trenchfoot, frostbite and immersion foot are virtually the same conditions, and are the result of ground type cold injury. Although the problem has been recognized since ancient times, and was described well during World War I, there were 91,000 cold injury cases recorded in World War II. In developing the history of experience with cold injury in each theater of World War II, a somewhat undesirable repetition of medical information results. Nevertheless, there is a good description of the pathogenesis, clinical picture, management and prevention. Also high altitude frostbite is well covered. The book is excellently illustrated.

CARLTON L. SEXTON

BOOKS RECEIVED FOR REVIEW

The Anatomy of the Nervous System. Its Development and Function, 10th edition. By STEPHEN WALTER RANSON AND SAM LILLARD CLARK. 622 pp., $9.50. W. B. Saunders Co., Philadelphia, Pa.

Basic Issues in Psychiatry. By PAUL V. LEMKAU. 106 pp., $3.50. Charles C Thomas, Springfield, Ill.

The Central Nervous System and Behavior. Transactions of the First Conference, Feb. 1958. Edited by MARY A. B. BRAZIER. Sponsored by The Josiah Macy, Jr. Foundation and The National Science Foundation. 450 pp., $5.25. The Josiah Macy, Jr. Foundation Publications, New York, N. Y.

Color Atlas of Morphologic Hematology with a Guide to Clinical Interpretation, revised edition. By GENEVA A. DALAND. Edited by THOMAS HALE HAM. 72 pp., plus 30 pp. of illustrations and legends, $6.75. Harvard University Press, Cambridge, Mass.

Current Therapy 1959. Edited by HOWARD F. CONN. 781 pp., $12.00. W. B. Saunders Co., Philadelphia, Pa.

Diseases of the Colon and Anorectum, Vols. I and II. Edited by ROBERT TURELL. 1238 pp., plus 68 pp. of index. $35.00 per set. W. B. Saunders Co., Philadelphia, Pa.

A History of Neurology. By WALTHER RIESE. Foreword by FELIX MARTI-IBANEZ. 223 pp., $4.00. MD Publications, New York, N. Y.

A History of Ophthalmology. By GEORGE E. ARRINGTON, JR. Foreword by FELIX MARTI-IBANEZ. 174 pp., $4.00. MD Publications, New York, N. Y.

Physical Diagnosis. The History and Examination of the Patient. By JOHN A. PRIOR AND JACK S. SILBERSTEIN. 388 pp., $7.50. The C. V. Mosby Co., St. Louis, Mo.

Radioactive Isotopes in Clinical Practice. By EDITH H. QUIMBY, SERGEI FEITELBERG AND SOLOMON SILVER. 451 pp., $10.00. Lea & Febiger, Philadelphia, Pa.

Urology in Outline. By T. L. CHAPMAN. 176 pp., $6.75. E. & S. Livingston, Edinburgh and London. The Williams & Wilkins Co., Baltimore, Md. exclusive U. S. agents.

SOME CLINICAL AND PHYSIOLOGIC ASPECTS OF ANGINA PECTORIS[1, 2]

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The memory of Dr. Thayer brings to mind the remark of Enobarbus about Cleopatra:

> "Age cannot wither . . . nor custom stale
> Her infinite variety . . ."

The thirty-nine years which have passed since, as a second-year student, I was first exposed to the variety and to the quality of Dr. Thayer as a person and as a teacher, have neither withered nor dimmed the vivid and lasting imprint he left upon his pupils. His contributions to medical literature require no elaboration before this audience. His opinion that he, as head of the department, should personally teach the introductory course in medicine set an example which many of us have followed, although less successfully, through the years. His meticulous bedside observation, his gentleness with patients, his kindness to students and his devotion to his profession, not only as a science and as an art, but also as a proud cultural tradition, were among the memorable qualities of this medical Sir Galahad. Among a remarkable group of teachers he was, in the mind of at least one worshipful student, the "noblest Roman of them all."

The heart had a prominent place in Dr. Thayer's broad clinical interests. His studies of the third sound offered the chance to utilize his astuteness in physical diagnosis. What a joy it would be to him could he know of the splendid work now being done on heart sounds here at Hopkins. His observations on bacterial endocarditis involved the combination of auscultation with that insight into the febrile diseases which was so characteristic of the Oslerian pupils. Since my own interest in the heart was first seriously aroused by Dr. Thayer, I am happy to offer respect to our memory of him by discussing some clinical and experimental problems related to this organ.

Heberden's angina pectoris may often be correctly diagnosed by its classical history. However, when the story is atypical the diagnosis may be very diffi-

[1] The investigations reported herein have been aided by grants from the Ingco Foundation, the Calhoun County Heart Association, the Claude Dorsey Memorial Fund and the U. S. Public Health Service (H-1912-C-4).

[2] Lecture I of the twenty-fourth course of lectures under the William Sydney Thayer and Susan Read Thayer Lectureship in Clinical Medicine. Delivered at The Johns Hopkins Hospital April 6, 1959.

cult. Angina of effort may be mimicked by various benign disorders which may also cause pain during exertion. Only the careless historian will be deceived by pleural pain, which is likely to be aggravated by the deeper breathing during exercise or by the patient who unconsciously sucks air into his stomach while walking and has instant relief when it is regurgitated. The excitable person who hyperventilates during effort and develops a feeling of tightness in the muscles of the chest (which may precede the classical carpal spasm) may present a problem. Even more difficult is the differentiation of angina from that type of pain which occurs on walking in patients with mitral stenosis or other conditions which may cause exertional elevation of the pulmonary arterial pressure. Indeed, there is some reason to believe that this pain arises in the right ventricle rather than in the pulmonary vessels, and that it may represent a type of angina which is often unresponsive to glyceryl trinitrate (nitroglycerin).

The most common variety of nonanginal exertional pain is that which arises in the chest wall or in the arms. Walking is attended by increased motion of these structures which may produce pain in consequence of various trivial disorders. The commonly associated local tenderness in the costochondral or chondrosternal articulations, in the xiphosternal junction or in the pectoral muscles is frequently overlooked. If the patient happens to also have a flattened or inverted T wave due to some entirely innocent disorder, he is likely to be the tragic victim of a false positive diagnosis of coronary disease.

The problem is rendered doubly difficult when the two disorders, angina pectoris and skeletal pain, coexist. This is frequent because the reflex sympathetic dystrophy which produces the well known shoulder-hand syndrome as the result of coronary disease also frequently causes pain in the structures of the thoracic wall. This secondary skeletal pain is even more common in the chest than in the arm. The demonstration that a middle-aged male or an elderly female has pain which is undoubtedly arising in these skeletal structures should lead to a meticulous search for angina pectoris, which may be masked by the coexisting innocent disorder. Only after thorough study is one justified in concluding that the skeletal pain is not secondary but primary and, therefore, unrelated to coronary disease. Such studies usually involve separation of the effects of increased heart work due to climbing stairs, while the local part is moving minimally from the effects of vigorous local motion when the patient is standing and the work of the heart is only slightly increased. An example of the procedure employed is shown in Table I.

There are many patients in whom the response to glyceryl trinitrate is of crucial diagnostic importance. Some of the pitfalls which may trap the unwary in this evaluation are indicated in Table II. Regardless of these difficulties and despite the progress which has been made in the application of objective methods, the clinical study of the pain itself, and especially of the circumstances

TABLE I

Mrs. K., Age 43, with Clinical and ECG Evidence of Infarct 4 Months Previously, Frequent Exertional Pain in Left Shoulder for 1 Month

On rare occasions substernal discomfort with exertion. Mild diabetes

ECG: No further change with exercise sufficient to produce pain

Procedure	Trials	Time of Onset of Pain: sec.	Pain in		Conclusion
			Arm	Chest	
Standard walking (steps)	6	105 ± 15	+	0	Arm pain
Standard walking (steps) after nitroglycerin	5	108 ± 20	+	0	Muscular (Secondary skeletal)
Vigorous swinging of arms					
Left	3	23 ± 8	+	0	
Right	3	73 ± 12	+	0	
Standard walking: hands on hips	3	160 ± 25	0	+	Substernal
Standard walking: hands on hips after nitroglycerin	3	270 ± 30	0	+	Pain anginal

TABLE II

Problems in the Interpretation of the Nitroglycerin Effect in Relieving Chest Pain

1. Relief of nonanginal pain:
   (a) Confusion with spontaneous relief—controls necessary.
   (b) Relief of nonanginal pain is rarely rapid and complete.
2. Failure to relieve anginal pain:
   (a) Inadequate dosage
   (b) Deterioration of drug } Necessity to induce headache or flush before drawing conclusions
   (c) Infarctional or preinfarctional pain
3. Undesirable effects:
   (a) Headache
   (b) Aggravation of postural hypotension
   (c) Development of tolerance

which precipitate and relieve it, remains the most valuable procedure in the diagnosis of angina pectoris.

Such a statement does not mean that objective methods of study are either useless or hopeless. There are some persons in whom the electrocardiogram yields decisive information when the history is inconclusive or even when the patient has had no pain at all. We have recently seen a patient who complained only of mild steady pain in the right upper quadrant, and slight exertional dyspnea. There was no story of angina or of myocardial infarction. Examination revealed minimal cardiac enlargement and a protodiastolic gallop. The electrocardiogram displayed deep inversion of T waves in V_3, V_4 and V_5. In this patient, as in many others, the electrocardiogram was of crucial importance in the recognition of coronary disease.

The realization of the great value of the ECG in diagnosis in *some* patients

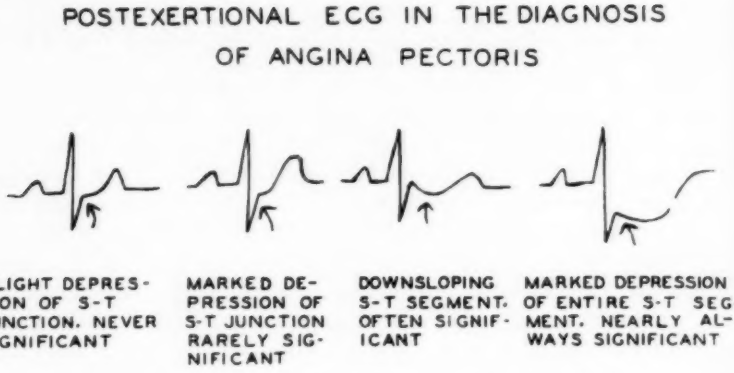
POSTEXERTIONAL ECG IN THE DIAGNOSIS OF ANGINA PECTORIS
SLIGHT DEPRESSION OF S-T JUNCTION. NEVER SIGNIFICANT
MARKED DEPRESSION OF S-T JUNCTION RARELY SIGNIFICANT
DOWNSLOPING S-T SEGMENT. OFTEN SIGNIFICANT
MARKED DEPRESSION OF ENTIRE S-T SEGMENT. NEARLY ALWAYS SIGNIFICANT

FIG. 1. The type of change illustrated in the left record is observed in many normal subjects following exertion. The greater depression, one to two millimeters, of the S-T junction occurring after exercise is often seen in patients with angina but is not excessively rare in persons who have no clinical evidence whatever of coronary disease. Therefore, the diagnosis in a patient presenting such a record must be based on other criteria.

In the absence of digitalis therapy the change shown in the third record when appearing after effort usually indicates myocardial ischemia. Thus far we have not encountered the transient extreme postexertional depression of the entire S-T segment as shown in the right figure in a patient who lacked evidence of coronary disease.

has led to the assumption that it should be of value in *all* instances. There are many causes of inverted T waves in healthy persons. Among the commonest are misplaced precordial electrodes, postprandial shifts in potassium, healed pericarditis, old contusions of the heart, anxiety and hyperventilation.

The majority of patients with angina pectoris have normal electrocardiograms at rest. There is a strong difference of opinion concerning postexertional abnormalities. I believe that the transient appearance after exercise of a markedly depressed or even of a down-sloping S-T segment is strong evidence for myocardial ischemia (Fig. 1). However, a depression of the S-T junction by one half to one millimeter, followed by an upward slope to a normal T peak, is not likely to be significant. Such a change, which we have observed in many normal subjects, must either be disregarded entirely or interpreted in the light of the associated clinical findings.

The difference of opinion which exists concerning the electrocardiograms applies also to the ballistocardiogram. It would be idle for me to discuss this question before an audience which includes those who have made important contributions to a subject which I have not studied in detail. My colleagues, who have worked extensively with the BCG, are in general agreement with the views which have been published by the Hopkins group.

Tennant and Wiggers showed in dogs that following occlusion of a coronary

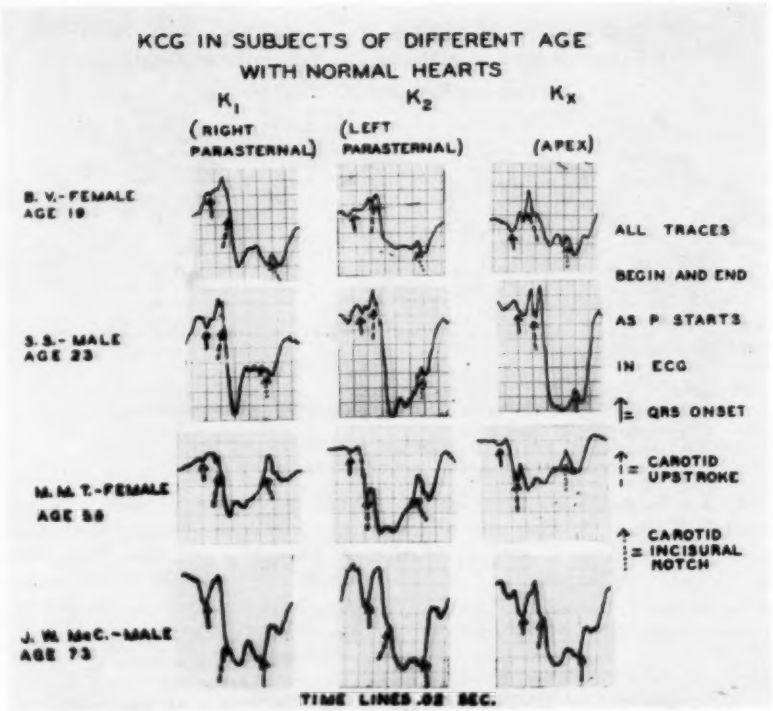

FIG. 2. During ejection all subjects display well marked inward motion at all areas. The apex beat (upstroke at K_4, starting immediately before the carotid upstroke) is less pronounced in the older than in the younger subjects. This is in accord with the common clinical observation of increasing difficulty in feeling the impulse in elderly persons with normal hearts. Despite this and other slight differences, it should be noted that the exaggerated atrial motions (i.e., movements starting before Q) and the pronounced midsystolic bulges so commonly seen over the left precordial area during anginal attacks (Figs. 3 and 4) are not observed in either young or old persons with normal hearts.

artery the ischemic area displayed systolic expansion instead of the normal contraction. Similar observations were made by Prinzmetal and his colleagues. We have repeatedly encountered systolic outward motion of the precordium in patients during anginal attacks. Sometimes the bulge is easily palpable but more often its detection requires tracings of precordial movement, which we designate "Kinetocardiograms." Examples of normal records and of tracings obtained from persons with coronary disease are shown in Figs. 2, 3, and 4 respectively.

Although these studies of precordial movements in patients with angina pectoris are far from complete, certain general conclusions appear to be emerg-

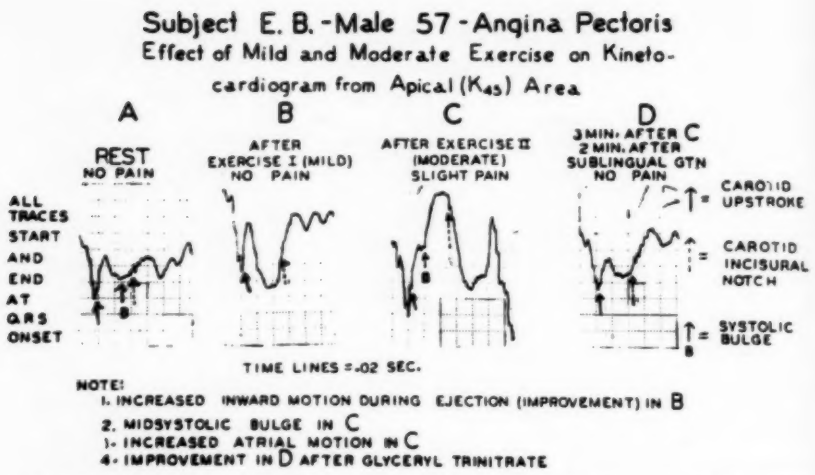
Subject E. B. - Male 57 - Angina Pectoris
Effect of Mild and Moderate Exercise on Kineto-cardiogram from Apical (K_{45}) Area
A
REST
NO PAIN
B
AFTER EXERCISE I (MILD)
NO PAIN
C
AFTER EXERCISE II (MODERATE)
SLIGHT PAIN
D
3 MIN. AFTER C
2 MIN. AFTER SUBLINGUAL GTN
NO PAIN
ALL TRACES START AND END AT QRS ONSET
CAROTID UPSTROKE
CAROTID INCISURAL NOTCH
SYSTOLIC BULGE
TIME LINES = .02 SEC.
NOTE:
1. INCREASED INWARD MOTION DURING EJECTION (IMPROVEMENT) IN B
2. MIDSYSTOLIC BULGE IN C
3. INCREASED ATRIAL MOTION IN C
4. IMPROVEMENT IN D AFTER GLYCERYL TRINITRATE

FIG. 3 A–D. A. Decreased inward movement during ejection (compare with normal subjects in Figure 2). B. After minimal exercise the record showed increased inward motion indicating improvement. C. After additional exercise typical anginal pain developed and a large midsystolic bulge appeared. At the same time the atrial motions (i.e. those deflections starting just before the next QRS) were sharply exaggerated. D. The bulge disappeared and the trace returned to its original state after a sublingual pill of glyceryl trinitrate.

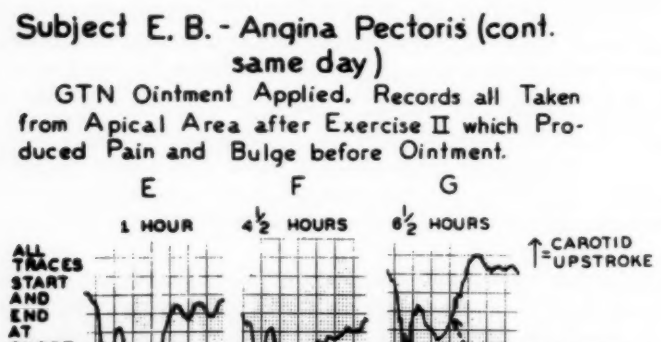
Subject E. B. - Angina Pectoris (cont. same day)
GTN Ointment Applied. Records all Taken from Apical Area after Exercise II which Produced Pain and Bulge before Ointment.
E
1 HOUR
F
4½ HOURS
G
6½ HOURS
ALL TRACES START AND END AT ONSET OF QRS
CAROTID UPSTROKE
CAROTID INCISURAL NOTCH
TIME LINES = .02 SEC.
NOTE THAT EXERCISE PRODUCES NO BULGE OR OTHER ABNORMALITY.

FIG. 3 E–G. The postexertional record remained normal for 6½ hours after the ointment was applied. In most subjects the duration of action of the ointment is three to four hours.

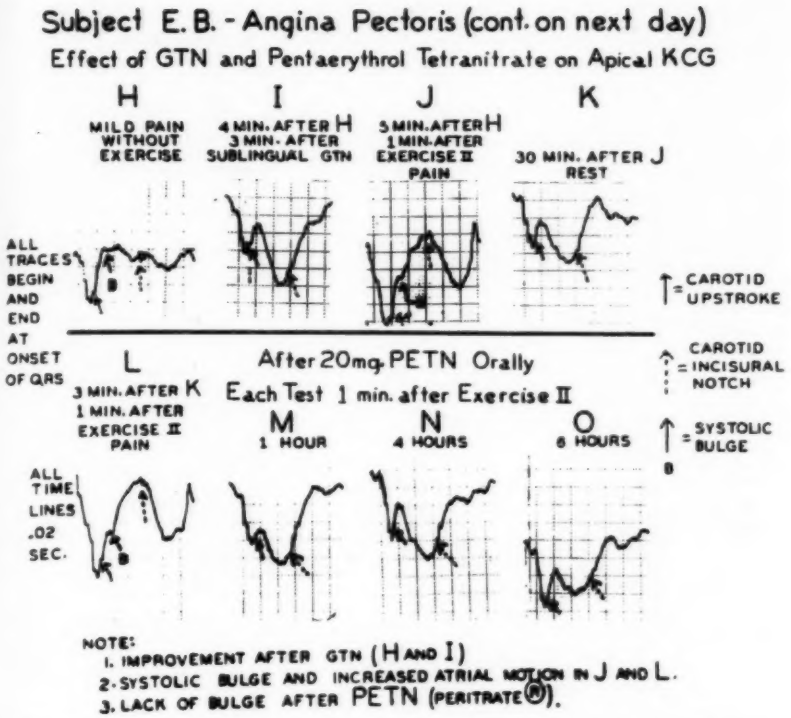
Subject E. B. - Angina Pectoris (cont. on next day)
Effect of GTN and Pentaerythrol Tetranitrate on Apical KCG
H
MILD PAIN WITHOUT EXERCISE
I
4 MIN. AFTER H 3 MIN. AFTER SUBLINGUAL GTN
J
5 MIN. AFTER H 1 MIN. AFTER EXERCISE II PAIN
K
30 MIN. AFTER J REST
ALL TRACES BEGIN AND END AT ONSET OF QRS
↑ = CAROTID UPSTROKE
CAROTID INCISURAL NOTCH
↑ = SYSTOLIC BULGE
L
3 MIN. AFTER K 1 MIN. AFTER EXERCISE II PAIN
After 20mg. PETN Orally
Each Test 1 min. after Exercise II
M
1 HOUR
N
4 HOURS
O
6 HOURS
ALL TIME LINES .02 SEC.
NOTE:
1. IMPROVEMENT AFTER GTN (H AND I)
2. SYSTOLIC BULGE AND INCREASED ATRIAL MOTION IN J AND L.
3. LACK OF BULGE AFTER PETN (PERITRATE®).

FIG. 3 H–O. (H) During a spontaneous attack of mild pain the normal inward motion of ejection was again replaced by a minimal systolic bulge. This disappeared after glyceryl trinitrate (I) but reappeared in exaggerated form after exercise (J) and again vanished after one half hour's rest (K).

The same exercise which previously produced pain and a well marked systolic bulge (L) had no such effect 1, 4 and 6 hours after pentaerythrol tetranitrate (M, N, O).

Postexertion pain (J and L) was again associated with exaggeration of atrial motions.

ing. When pain is absent the records may be normal or abnormal. During anginal attacks the great majority of patients—more than ninety per cent of those studied thus far—display abnormalities. One of these is the systolic bulge which usually appears during the early part of ejection, and partially or completely replaces the normal inward motion (Figs. 3 and 4). This phenomenon is believed to be due to loss of contractile power and consequent passive ballooning of flabby ischemic ventricular muscle. Such a bulge is present during anginal seizures in most patients, and may antedate the pain (Figs. 3W and 4A).

Another abnormality which is less frequently observed during attacks is

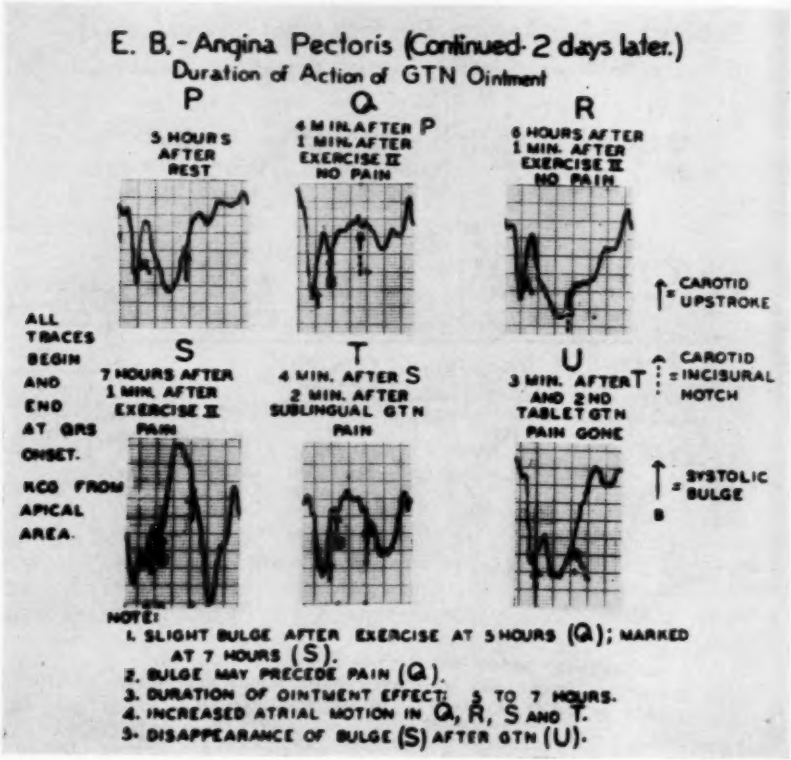
E. B. - Angina Pectoris (Continued - 2 days later.)
Duration of Action of GTN Ointment
P
5 HOURS AFTER REST
Q
4 MIN. AFTER P
1 MIN. AFTER EXERCISE II
NO PAIN
R
6 HOURS AFTER
1 MIN. AFTER EXERCISE II
NO PAIN
↑ = CAROTID UPSTROKE
ALL TRACES BEGIN AND END AT QRS ONSET.
KCG FROM APICAL AREA.
S
7 HOURS AFTER
1 MIN. AFTER EXERCISE II
PAIN
T
4 MIN. AFTER S
2 MIN. AFTER SUBLINGUAL GTN
PAIN
U
3 MIN. AFTER T AND 2ND TABLET GTN
PAIN GONE
A = CAROTID INCISURAL NOTCH
B = SYSTOLIC BULGE
NOTE:
1. SLIGHT BULGE AFTER EXERCISE AT 5 HOURS (Q); MARKED AT 7 HOURS (S).
2. BULGE MAY PRECEDE PAIN (Q).
3. DURATION OF OINTMENT EFFECT: 5 TO 7 HOURS.
4. INCREASED ATRIAL MOTION IN Q, R, S AND T.
5. DISAPPEARANCE OF BULGE (S) AFTER GTN (U).

FIG. 3 P–U. At 5 hours after the ointment a minimal systolic bulge appeared after effort (P and Q). The six-hour postexertional record remained normal (R), but seven hours after the inunction the previously observed gross abnormalities (bulge during ejection and exaggerated atrial movements) appeared (S). On this occasion two sublingual tablets of glyceryl trinitrate were required to relieve the pain and to overcome entirely the abnormalities in the KCG (T and U).

increase in the motions which begin during the P-R interval, and which are therefore assumed to be related to atrial contraction (Figs. 3 and 4). Although direct evidence is lacking, it seems probable that this exaggeration of atrial force is related to increase in ventricular residual blood, with elevation of end diastolic pressure and greater distention of the thin-walled auricles. If this conclusion is correct, the records afford an excellent demonstration in the human subject of the validity of Starling's law of the heart as applied to the atrium. We have often observed abnormally large atrial motions in patients with heart failure due to various disorders. It is likely that the exaggeration after exercise will ultimately be proved to be related to weakness of the ven-

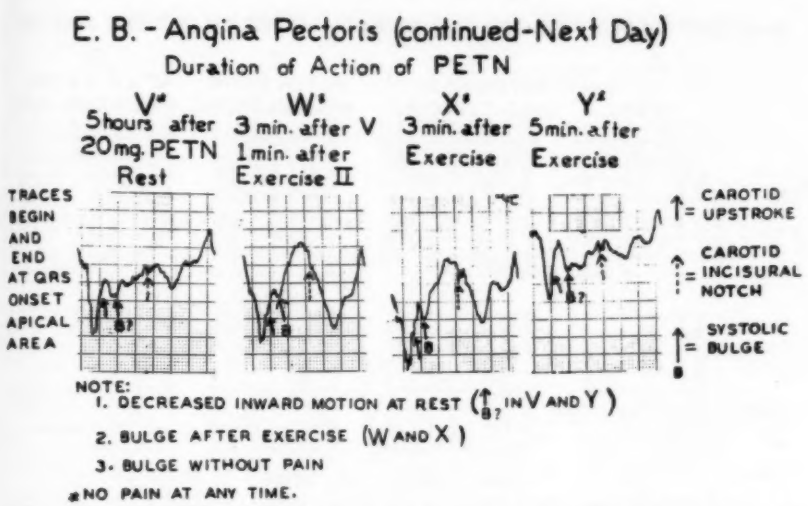
E. B. - Angina Pectoris (continued-Next Day)
Duration of Action of PETN
V*
5 hours after 20 mg. PETN
Rest
W*
3 min. after V
1 min. after Exercise II
X*
3 min. after Exercise
Y*
5 min. after Exercise
TRACES BEGIN AND END AT QRS ONSET APICAL AREA
CAROTID UPSTROKE
CAROTID INCISURAL NOTCH
SYSTOLIC BULGE
NOTE:
1. DECREASED INWARD MOTION AT REST (↑B? IN V AND Y)
2. BULGE AFTER EXERCISE (W AND X)
3. BULGE WITHOUT PAIN
*NO PAIN AT ANY TIME.

FIG. 3 V–Y. The control record (V), taken 5 hours after pentaerythrol tetranitrate, exhibited decreased inward motion during ejection with a questionable slight midsystolic bulge. Exertion caused a marked bulge and exaggerated atrial motion (W). These abnormalities decreased during the next few minutes (X and Y) but did not disappear entirely. In this instance exertion produced KCG abnormalities without pain.

Comparison of these traces with the previous ones (P, Q, R) suggests that the ointment was superior to the oral preparation in terms of duration of action.

tricular musculature from any cause rather than to angina pectoris *per se*. More observations on this point are needed.

Various other abnormalities of the kinetocardiogram may be observed during anginal attacks but the two which have been mentioned are the only ones which thus far have been studied in any detail. We do not as yet know whether these changes are sufficiently constant and specific to be of any great diagnostic value. The limits of variation at rest and after exercise must be clearly defined for a large series of older persons with normal hearts before one ventures to utilize the kinetocardiogram in diagnosis.

Additional evidence for impairment of the contractile process during anginal attacks may sometimes be observed in carotid pulse traces. Even when every allowance is made for the technical problems inherent in securing reproducible carotid tracings, we still find that the occurrence of a precordial bulge is frequently associated with a momentary systolic dip in such records (Fig. 5). Similarly, the BCG may display a delayed headward peak, appearing after the onset of the normal J-K downstroke. The myocardial energy which is utilized in distending the ischemic area seems to be subtracted from that normally available for expelling blood. Thus the anginal episode appears to have a

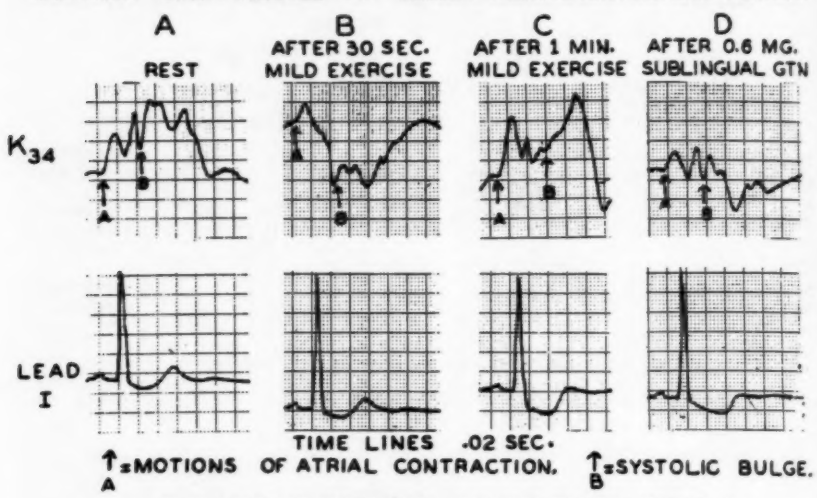

FIG. 4. KCG recorded from the fourth interspace in the V_3 line.

Minimal exercise caused no change in the ECG but produced definite improvement in the precordial tracings, as shown by decrease in the systolic bulge and in the magnitude of the atrial motions (Compare B with A).

When the exercise was repeated at the same rate but for twice as long a time, there was pronounced decline in the S-T segment, marked exaggeration of the atrial movements, and the midsystolic bulge became very prominent (C). Nitroglycerin caused reversal of these three phenomena (D).

In this patient both the ECG and the KCG displayed abnormalities at rest, and exercise which was not sufficient to produce pain caused striking increase in these abnormalities (C). The improvement by mild exercise (B) was readily apparent in the KCG but was not detected by the ECG.

double effect on ventricular action. Aside from the impairment of contraction in the ballooning region, there is reduction in the ejection effectiveness of the healthy myocardial fibers.

The effect of measures aimed at the relief of myocardial ischemia on precordial motions has been studied. On many occasions we have seen the records change toward normal as the pain vanishes, following sublingual glyceryl trinitrate. Of special interest is the study of certain long-acting coronary dilators. Following inunction with an ointment containing glyceryl trinitrate, the abnormality previously produced by exercise may be absent for several hours. Recently, we have begun to compare the effect of this preparation (Nitrol® ointment) with pentaerythrol tetranitrate (Peritrate®), an oral prepa-

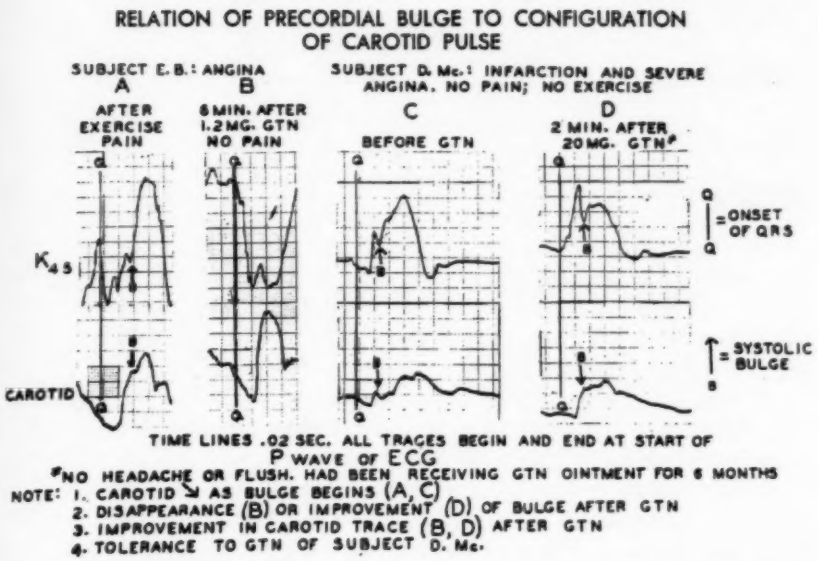
RELATION OF PRECORDIAL BULGE TO CONFIGURATION OF CAROTID PULSE
SUBJECT E. B.: ANGINA
A
AFTER EXERCISE PAIN
B
6 MIN. AFTER 1.2 MG. GTN NO PAIN
SUBJECT D. Mc.: INFARCTION AND SEVERE ANGINA. NO PAIN; NO EXERCISE
C
BEFORE GTN
D
2 MIN. AFTER 20 MG. GTN*
K45
CAROTID
= ONSET OF QRS
= SYSTOLIC BULGE
TIME LINES .02 SEC. ALL TRACES BEGIN AND END AT START OF P WAVE OF ECG
*NO HEADACHE OR FLUSH. HAD BEEN RECEIVING GTN OINTMENT FOR 6 MONTHS
NOTE: 1. CAROTID ↘ AS BULGE BEGINS (A, C)
2. DISAPPEARANCE (B) OR IMPROVEMENT (D) OF BULGE AFTER GTN
3. IMPROVEMENT IN CAROTID TRACE (B, D) AFTER GTN
4. TOLERANCE TO GTN OF SUBJECT D. Mc.

FIG. 5. When the precordial bulge was pronounced (A, C) the configuration of the carotid pulse trace was slightly (A) or grossly (C) abnormal. Both records improved after sublingual glyceryl trinitrate (B, D). Subject EB, who had been receiving this drug for a few days only, displayed the usual sensitivity to it. However, the amount required to produce an effect in subject D. Mc. was extremely large. This patient had been under treatment with the GTN ointment for several months and had developed marked tolerance. He subsequently was able to take 30 mg. sublingually without headache or flushing. Later when the drug was discontinued his angina became even more severe but after some weeks he was again responsive to 0.6 mg.

ration with prolonged action. Both drugs can be readily demonstrated to exert a favorable effect (Fig. 3), but many more patients will need to be studied before a definitive statement can be made that one preparation is better than the other. It may be that our present impression of the superiority of the ointment will prove to be erroneous. In any event, these observations indicate that in patients with angina pectoris both drugs may exert a prolonged favorable action not only on the pain but also on the contraction of the heart. Their beneficial effect must be balanced against certain drawbacks (Table III). Initially, the dosage and frequency should be rapidly increased until resting attacks have ceased. Then in order to prevent the development of tolerance (Fig. 5D), the amount of the long-acting dilator should be gradually reduced during a period of several weeks. Abrupt withdrawal may be hazardous. The management is aided by a diary recorded by the patient and indicating the exact time of pain in relation to the administration of the drug. In the absence

TABLE III

Use of Nitroglycerin Ointment to Prevent Anginal Attacks

Indications: Frequent attacks at rest, especially nocturnal; status anginosus; preinfarctional angina.
Dosage: ½ to 3 inches; slightly less than the headache dose.
Administration: Spread thinly on chest, abdomen or thighs (do not rub) every 3 to 6 hours.
Nocturnal angina: Apply at bedtime and about 4 hours later if necessary to prevent attacks.
Disadvantages: (1) Sudden withdrawal may precipitate numerous attacks.
(2) Prolonged administration may cause tolerance. Reduce dose by about 10 to 20 per cent per week, unless attacks of pain again become frequent.

of a definite precipitating factor, pain appearing at the height of the action (one to two hours after taking) calls for larger dosage, while attacks occurring as the effect declines (three or more hours after taking) suggests the need for more frequent administration.

We have seen many patients whose attacks are reduced by 90 per cent or more within twenty-four hours by the use of nitroglycerin ointment applied every two to six hours. The usual dose is one to three inches of the column as squeezed from the collapsible containing tube. The preparation should be spread over as wide an area as possible, but not rubbed. In most instances the proper dose is that amount which is not quite enough to cause headache.

Heberden mentioned the favorable effect of muscular effort: "I know one who set himself a task of sawing wood for half an hour every day, and was nearly cured." About thirty years ago Dr. Lewis Connor advocated regular mild exertion. More recently there have appeared a number of clinical and experimental studies pointing toward the favorable effect of exercise on the prevention of functional impairment of the coronary circulation. For some years it has been my custom to advise patients with angina to follow a sublinqual pill of glyceryl trinitrate with a short walk several times a day, the speed and distance being so regulated that the pain threshold is not exceeded. This advice has been based mainly on theoretical considerations, but a large number of patients have developed what appeared to be an increased exercise tolerance. Only recently has the problem been approached by objective methods. The number of patients thus far studied is small. Many of them have records which are normal at rest. In these persons improvement in the kinetocardiograms after mild exercise could not be anticipated and has not been found. However, some anginal patients display abnormal records at rest and may exhibit definite improvement with mild exercise, even though greater effort intensifies the original abnormality (Figs. 3A, B and C and Fig. 4). We also have noted that an exercise which previously made a precordial tracing more abnormal may have the reverse effect during the action of a coronary dilator drug.

Information is lacking concerning the frequency of occurrence of objective evidence of improvement by mild exertion. The most that can be said at present

is that exercise does have a favorable effect in *some* patients. This apparent paradox can be explained readily, provided one makes the assumption that in such persons the initial effect of exertion is to cause a greater increase in coronary flow than in cardiac work (Fig. 6). Unfortunately, there is at present no way to test this assumption directly in the anginal patient without incurring a serious hazard. It is only an hypothesis.

We may now summarize the preceding discussion and at the same time consider briefly some general principles of treatment of Heberden's disorder, and particularly of those patients who have repeated severe attacks at rest. The most immediate concern is that of preventing attacks of pain. Physical exertion should be limited to that which does not cause pain. Large meals and emotional disturbances, even of minor nature, are especially important. One frequently observes patients who have severe attacks following minimal psychic stress but can walk several hundred yards slowly without untoward effect and, indeed, with apparent benefit. Less commonly, cold air, cold sheets and even cold drinks precipitate attacks. Ectopic tachycardia and spontaneous hypoglycemia are rather rare inciting factors.

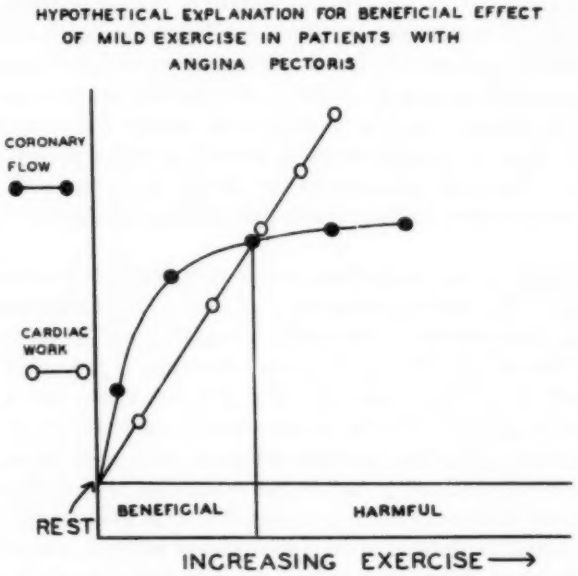

FIG. 6. When other factors are constant the flow of liquid through a tube tends to vary with the fourth power of the radius (Poiseuille's equation). Thus a slight degree of coronary dilatation, if induced by exercise, would have a large effect on flow. However, the work of the heart tends to increase in a relatively linear fashion. If this hypothetical concept is valid, exercise should be beneficial or harmful according to its degree in relation to the intersection of the two lines.

The second aim is the prevention of myocardial infarction and there is a growing body of evidence that long-term anticoagulant thereby is of value for this purpose. However, the question is not yet completely settled.

The third concern is that of preventing further narrowing of the main coronary branches. There is nothing I could say on this subject which involves diet, nicotinic acid and possibly estrogens that has not been better said by others.

The final principle is the attempt to widen the collateral coronary channels. Quick-acting dilators to relieve the pain and long-acting dilators to prevent it are of crucial importance here. The evidence which has been cited indicates that coronary dilator drugs may not only relieve and prevent pain but may promote more effective ventricular contraction.

There is also reason to believe that minimal physical exertion, always less than that which induces pain, is beneficial in some patients. It would appear that such exercise is particularly effective in dilating the collaterals when taken either immediately after a sublingual tablet or at the height of action of a long-acting coronary dilator drug.

The uncertainty which is a cardinal feature of angina pectoris, operates in both directions. A person who is progressing favorably may suddenly expire. However, another patient who has numerous severe and prolonged attacks at rest for many weeks or even for months, and who has had multiple infarction, may make an almost complete recovery and survive for years in complete comfort. We have no certain means of foretelling which patient will pursue which course. Thorough attention to the details of therapy will often bring prolonged improvement to the patient and abiding satisfaction to the physician.

These are some of the compelling aspects of Heberden's fearsome "disorder of the breast." The electrocardiogram and the ballistocardiogram have each made certain contributions to the earlier recognition and to the better understanding of this malady. It is our hope that the study of precordial movements will represent an additional advance. But it is not likely that a disorder so subtle as angina pectoris will ever be detectable in all instances by any instrumental procedure. Objective methods will be of diagnostic import in certain patients, and will continue to aid in the evaluation of the efficacy of treatment. However, the numerous tiny myocardial scars so often seen after death indicate that in some persons at least the areas of ischemia responsible for the pain are very small. When we reflect on the intensity of toothache in relation to the size of the tooth pulp, it seems probable than anginal pain may sometimes arise from minute areas of tissue. It is highly unlikely that such small localized ischemic islands will invariably produce clinically detectable electrical or mechanical alterations in the functions of the heart. If this reasoning is

sound there will always remain instances which defy recognition by objective methods. In such patients a correct decision will continue to depend on the meticulous history and the careful observation of those circumstances which provoke and relieve the pain. This is certainly true at present and will probably remain so in the future. Dr. Thayer's often expressed opinion that quantitative studies should complement but not supplant bedside observation would appear to be just as valid now as a half century ago. In this respect age has not withered nor custom staled the soundness of his judgment.

MOVEMENTS OF THE HEART AND VELOCITY OF PRESSURE CHANGE DURING THE FIRST PART OF SYSTOLE[1, 2]

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Early in their professional lives, Dr. Thayer's pupils learned from him that the study of the works of the medical giants of the past may be a source, not only of deep emotional satisfaction but also of strong intellectual stimulation. As an example we may consider William Harvey.

Longer ago than one likes to admit, I devoted some years to the study of the various types of circulatory failure. As these investigations were nearing completion and were being assembled into a monograph, I chanced to re-read Harvey's book. In a few lines he described how the heart of a snake became pale and empty when the vena cava was blocked but suffused and distended when the aorta was occluded. He then went on to say that this was evidence of two kinds of death—failure from a lack and suffocation from an excess.

The initial impact of this passage from Harvey was disturbing to the young investigator. Here in black and white were his cherished concepts of backward and forward failure clearly described three centuries before.

On further reflection disappointment was supplanted by satisfaction. If the concepts were less original than had been thought they had at least already received confirmation and vindication by one who spoke with the voice of authority. Furthermore, it was gratifying to feel that even in a most minor way one could participate in a chain of thinking which originated with the father of modern scientific medicine. Finally, there was the thought that perhaps my one time playmate and later work mate, Alfred Blalock, might not be entirely in error in his exemplification and advocacy of the virtue of humility. The purchase of even a few milligrams of wisdom at the price of many liters of deflated ego represents a good bargain.

Since that experience the works of Harvey have been even more intriguing. I have often wondered why he chose "De Motu Cordis" as the initial phrase in the title of his book. Certainly he realized that it was the circulation of the blood which was the most significant part of his discovery. Perhaps the reason is to be found in these lines:

[1] The research herein reported was aided by grants from the Ingco Foundation, the American Heart Association, the Claude Dorsey Memorial Fund and the U. S. Public Health Service (H-1912 C) (4).

[2] Lecture II of the twenty-fourth course of lectures under the William Sydney Thayer and Susan Read Thayer Lectureship in Clinical Medicine. Delivered at The Johns Hopkins Hospital April 7, 1959.

"When I first tried animal experimentation for the purpose of discovering the motions and functions of the heart by actual inspection and not by other people's books, I found it so truly difficult that I almost believed with Fracastorius that the motion of the heart was to be understood by God alone. I could not really tell when systole or diastole took place, or when and where dilatation or constriction occurred, because of the quickness of the movement. In many animals this takes place in the twinkling of an eye, like a flash of lightening." (Leakes' translation)

Perhaps Harvey was especially fascinated by the movements of the heart because they are so complex and so difficult to interpret. Since World War II, important investigations of heart movements in animals have been made by Rushmer, and in the human subject by numerous investigators who have applied the electrokymographic method. Precordial motions have been studied by Dressler, by Luisada, and by our group in Birmingham. However, there is still much to be learned about the motion of the normal and the diseased human heart. Those problems have constituted my special interest during the past ten years. We have been particularly concerned with the sequence of contraction in the various portions of the ventricular muscle, and my discussion today is primarily concerned with this question.

About eight years ago, while attempting to secure anteroposterior ballistocardiograms, we happened to obtain traces from the right and left sides of the sternum and found that they were strikingly different. Further observation indicated that normal subjects display a characteristic pattern of motion in the right and left parasternal areas and in the region of the apex (Fig. 1). These traces were grossly abnormal in many patients with cardiac disease. The next question was whether the study of these movements which we call kinetocardiograms would yield information of clinical value.

In the previous lecture it was shown that an ischemic area of the myocardium may display a characteristic systolic bulge which may disappear as the anginal attach subsides or which may persist for many months following an infarction. Today we may consider a few other patterns which appear to have some diagnostic value.

My colleague, Dr. E. E. Eddleman, has shown that the kinetocardiogram may be of aid in determining the relative degrees of stenosis and of insufficiency of the mitral valve. He and Dr. Duke Thomas have also shown that patients with right ventricular hypertrophy due to a flow load have kinetocardiograms which differ in a characteristic fashion from the traces associated with a pressure load.

Dr. John Jeffries has demonstrated, as did previous workers, another practical application of precordial traces. In a patient presenting a systolic murmur, a diastolic rumble and a third heart sound, the distinction between the opening snap of mitral stenosis and the filling gallop of regurgitation may be of

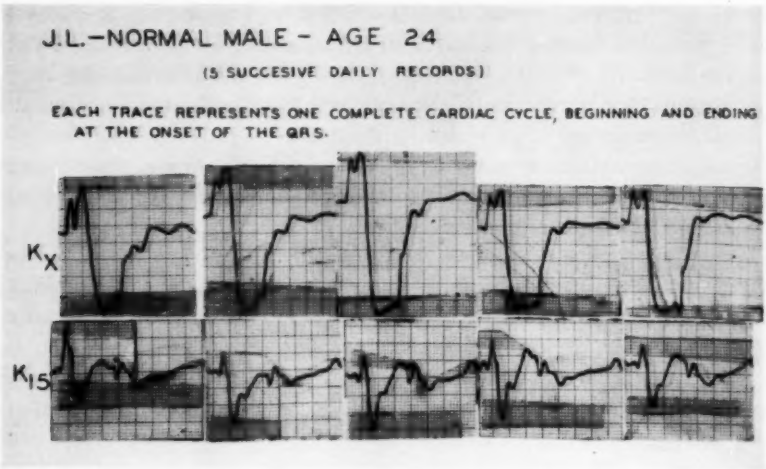
J.L.—NORMAL MALE - AGE 24
(5 SUCCESIVE DAILY RECORDS)
EACH TRACE REPRESENTS ONE COMPLETE CARDIAC CYCLE, BEGINNING AND ENDING AT THE ONSET OF THE QRS.
K_X
K_{15}

FIG. 1. The time lines are .02 second. The K_X trace is from the point of maximal impulse (apex) as located by palpation. The K_{15} record is from the right parasternal region and fifth interspace. The traces from the two different regions are entirely different but those from the same point on successive days are very similar.

At .04 second after Q there is a small inward motion (downstroke) in the K_{15} trace, while the K_X record moves outward. These deflections are not seen in all normal subjects.

The K_{15} trace displays an upstroke starting .06 second after the beginning of excitation. This motion is practically always seen in young subjects with normal hearts. It is often superimposed on and may be confused by a preceding gentle upslope which may start either before (4th complex) or after (1st complex) the QRS begins.

At .07 second after Q, inward motion is seen in the apical region. This movement also is constant in healthy persons and occurs at .06 $\pm$ .015 second after Q. Its magnitude is very variable in different subjects.

Approximately .09 second after Q there is sharp inward motion in the K_{15} trace and simultaneous outward movement at the apex. These are, respectively, related to volume change and recoil as ejection starts. Because the right ventricle usually starts to eject slightly before the left these motions are biphasic, as seen in the left K_X record and in all of the K_{15} traces.

compelling importance in deciding to perform or withhold valvulotomy. The opening snap occurs as filling starts while the gallop occurs later. Precordial tracings appear to represent the simplest accurate method of determining when filling begins in the human heart and are thus helpful in this decision.

Most instances of left ventricular hypertrophy are readily recognized by feeling the characteristic exaggeration of the localized apical thrust. Precordial tracings show that aside from this finding there is often an exaggerated backward pull in the parasternal regions prior to ejection.

Under these various conditions the kinetocardiogram appears to have some clinical value. However, the extent of its usefulness has not yet been deter-

mined, and frequently one can only say that the trace is grossly abnormal and consistent with certain physiologic alterations. Thus in many patients we must turn to other more complex and more expensive techniques—such as cardiac catheterization—for definitive diagnostic information. This limitation is due in some measure to our lack of knowledge concerning the mechanism of the normal movements of the heart and to our ignorance of the range of normality. Here we may draw an analogy with the electrocardiogram. Because of the long delay between the introduction of this instrument and a clear understanding of the variations which may occur in healthy subjects we have an appalling situation. As was indicated in the previous lecture, many thousands of persons are being subjected to unnecessary restrictions, economic deprivation, and needless anxiety because T-wave inversions or S-T junctional depressions of innocent nature are being misinterpreted as due to coronary disease.

If the kinetocardiogram is to be of value as an index of cardiac motion we must be certain that the limits of normality are accurately defined and that the mechanisms of the normal deflections are understood. It is this problem which I would like to discuss in some detail. Because the movements are numerous, complex, and different over various areas of the chest we shall confine ourselves to a single phase of the cardiac cycle, i.e., that between the onset of excitation and the beginning of the carotid upstroke. We may call this the pre-ejection phase of ventricular systole. It is almost but not quite identical with Wiggers' phase of isometric contraction which begins a few hundredths of a second after the QRS starts.

In normal subjects the duration of the pre-ejection phase of systole is slightly different in the two ventricles because the right usually ejects first. Thus the rises in pulmonary arterial and aortic pressures begin about .08 and .10 second, respectively, after the start of the QRS. This is well shown in the kinetocardiogram. These records almost always display inward motion of the right parasternal region and a quick outward movement (foreward recoil) of the left precordial area as the pulmonary pressure starts to rise. About 0.02 second later there is a leftward forward recoil movement of the apical region when the left ventricle ejects (Fig. 1). This occurs .02 $\pm$ .01 second before the start of the carotid upstroke. Thus the kinetocardiogram appears to offer a simple method of studying the effect of physiologic variables and of drugs on the duration of the isometric period of the two ventricles in normal human subjects.

After the start of excitation but before ejection there may be seen several precordial motions. The first of these is usually forward. It is observed after isolated P waves in persons with complete atrioventricular dissociation (Fig. 2). In normal subjects with slow rates and with relatively long P-R intervals this movement starts before Q, while it may follow Q when this interval is short. Therefore, this small forward motion is probably of atrial origin.

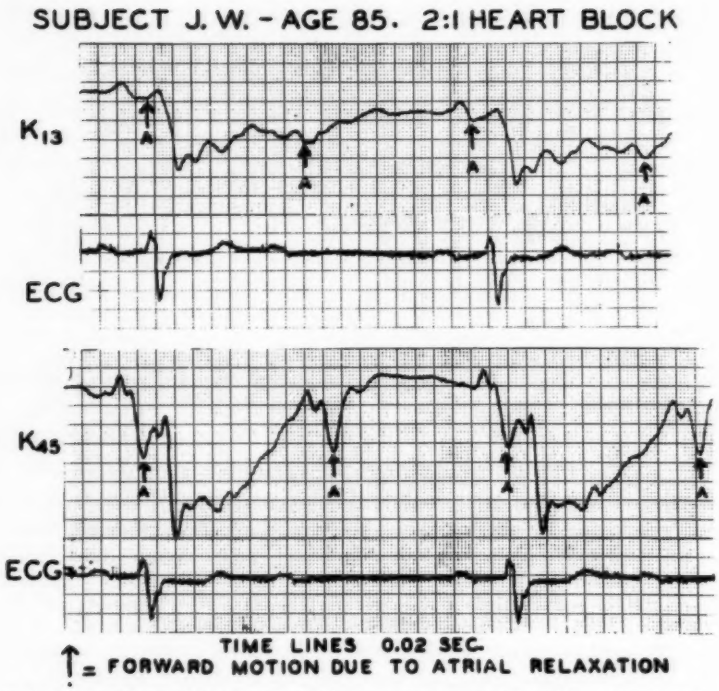

FIG. 2. The motions which are of atrial origin can be established by observing the trace following isolated P waves.

Following the conducted beats the motion ascribed to atrial relaxation occurs after the onset of the QRS in the K_{45} trace. This movement is clearly not of ventricular origin because it is seen after the isolated P waves.

In the K_{13} trace the terminal atrial forward motion starts either after—first complex—or before—second complex—the start of ventricular excitation.

About .03 second after the onset of excitation there may be a tiny backward motion in the right parasternal area associated with forward movement (occasionally backward) of the left precordium. These small deflections are not seen in all normal persons. Since they are sometimes present in patients with auricular fibrillation (Fig. 3) they must be of ventricular origin. The kinetocardiogram offers little information concerning their genesis.

At about .06 second after the start of the QRS two different motions may occur. One of them is forward and is often best seen in the right parasternal area (Fig. 1). This movement is usually exaggerated in persons with right ventricular hypertrophy. Hence we might conclude that it is related to contraction of the right ventricle. However more direct evidence concerning this matter is desirable.

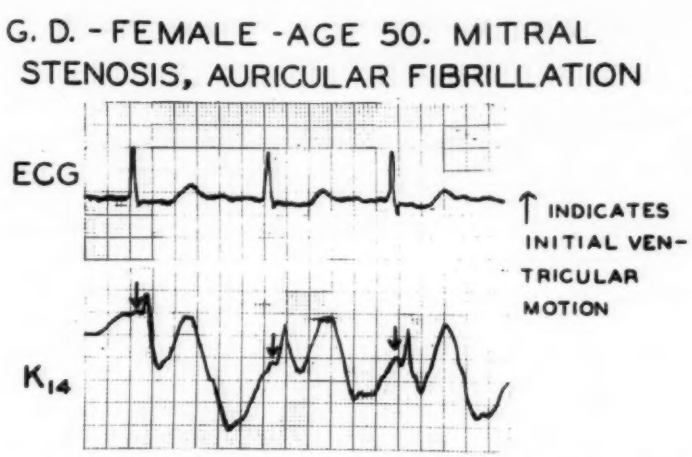

FIG. 3. Time lines .02 second. The small initial downstroke (arrow) is present in each complex at .04 second after Q. Since the atria are not contracting, the movement is clearly of ventricular origin. This motion is often absent, both in normal subjects and in patients with auricular fibrillation.

While the right parasternal area moves forward the region to the left of the sternum may retract (Fig. 1). Although the two events occur at about the same time either may precede the other. This backward motion is often much exaggerated in persons with left ventricular hypertrophy. We might, therefore, ascribe it to contraction of some portion of this chamber or of the septum, which appears to function as a part of the left ventricle. The frequent absence of this motion in patients with electrocardiographic evidence of septal infarction would support the idea that contraction of the septum is responsible. Once again, more direct evidence is needed.

This dilemma has occupied a major portion of my time during the past seven years. The conviction that one must understand the normal precordial movements before becoming too much concerned with abnormal motions has been continually frustrated by the realization that such interpretations as could be derived were based on indirect evidence. Only recently, and due to the efforts of my colleagues rather than of myself, has a somewhat better approach to this difficulty become available.

Drs. Joseph Reeves, Lloyd Hefner, William Jones, John Neal and Mr. Andrew Spear—the physicist of our group—have developed a method for studying in detail the velocity and the acceleration of pressure change. Having had nothing whatever to do with the initiation or perfection of this technique, I feel no embarrassment in praising it in terms of its probable future value as an investigative tool. Even slight shape changes in conventional pressure pulse

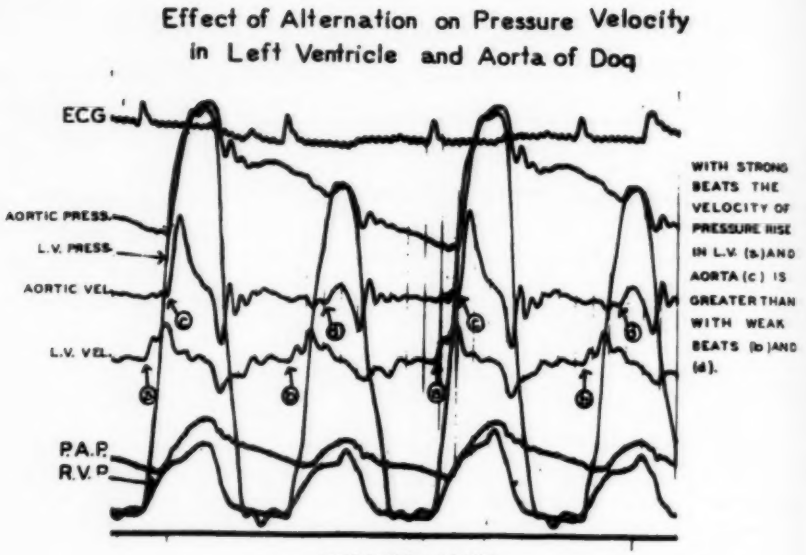
Effect of Alternation on Pressure Velocity
in Left Ventricle and Aorta of Dog
ECG
AORTIC PRESS.
L.V. PRESS.
AORTIC VEL.
L.V. VEL.
P.A.P.
R.V.P.
WITH STRONG BEATS THE VELOCITY OF PRESSURE RISE IN L.V. (b) AND AORTA (c) IS GREATER THAN WITH WEAK BEATS (b) AND (d).
TIME LINES = .04 SEC.

FIG. 4. The figure illustrates several points: 1. The velocity traces display much more detail than the customary pressure traces, because every slope change in the latter is reflected in the former by a directional alteration. 2. The difference in strength of the beats, which produces only moderate changes in the absolute pressures and in the ventricular velocity trace, is shown in a much more pronounced way in the aortic velocity traces. 3. The velocity of pressure rise in the left ventricle is not uniform but exhibits a decline shortly before ejection (See Figs. 7 and 8; Tables I and II). 4. The aortic velocity trace displays in each complex a small decline immediately before the sharp rise of ejection starts (See Fig 8 and Table 3).

records become directional changes in the velocity traces which, therefore, offer more detailed information.

We may now consider briefly some of the results which our group has obtained using this method, and then dwell in more detail upon its possible aid in clarifying the movements of the heart.

Drs. Reeves, Hefner and their associates have shown in dogs that the velocity of pressure rise in the ventricle during isometric contraction is directly related to myocardial contractility (Fig. 4), provided due allowance is made for the effects of the other major variables such as end diastolic pressure and end diastolic volume. They have learned that this relationship appears to hold true in the human subject as regards the velocity of pressure rise in the central aorta both at rest and during exercise. We, therefore, have hope that velocity pressure pulses from peripheral arteries may have value as an index of myocardial function. Whether it will ever be possible to obtain such information

from velocity traces of the carotid pulse without arterial puncture is still unknown.

Dr. William Jones and his coworkers have modified the catheter tip method of Fry for converting pressure to flow. In a given artery the velocity of flow may be obtained from the first derivative of the pressure pulse by Fry's electronic computer. The advantage of this technique is that only one catheter manometer system is required. A single measurement of cardiac output by the Hamilton dilution technique furnishes the necessary constants for obtaining absolute stroke volumes. Thus one can pass a catheter into the ascending portion of the aorta and measure continuously the output per beat during exercise or operation.

It would be inappropriate for me to discuss in detail the ballistocardiogram here at Hopkins, where so much of the important work of the past decade has been done. However, one point may be mentioned. Drs. Thomas, Frederick and Eddleman have shown that the deflections of the BCG are related not only to the flow of blood in the greater and lesser circuits but also to certain intracardiac forces. The second derivative, or acceleration of the carotid flow trace, has rather striking resemblances to the ballistocardiogram. Fig. 5 illustrates the conversion of the first derivative of the carotid volume pulse to a velocity flow curve which has been differentiated to obtain a reasonable approximation of the acceleration of flow in the central aorta. Perhaps such records will be of value in separating the intracardiac and the right ventricular forces from those which are related to the expulsion of blood by the left ventricle. More information concerning this point is needed.

For some years we have been attempting to secure at cardiac catheterization valid traces of velocity and acceleration of pressure change. These efforts have been relatively unsuccessful because of lags and also of artifacts due to catheter "whip." However, Dr. Leo Hughes has been able to surmount some of the difficulties, and we hope that with further elimination of technical defects such traces may be accurately recorded. Whether they will be of research value only or will yield practical information beyond that now obtainable from routine pressure traces is as yet unknown.

The technical problems involved in obtaining derivatives of pressure traces from the human heart in the operating room have been partially overcome by Drs. Duke Thomas and Earle Shugerman. We are now securing such records routinely but some years will elapse before their investigative or clinical value will be known.

These are some of the studies which my colleagues are pursuing with the velocity and acceleration of pressure change. My own work with this technique has been confined to an inquiry into the bearing which such records may have on the problem of heart movements and especially those of healthy human subjects. We may now turn our attention to this question.

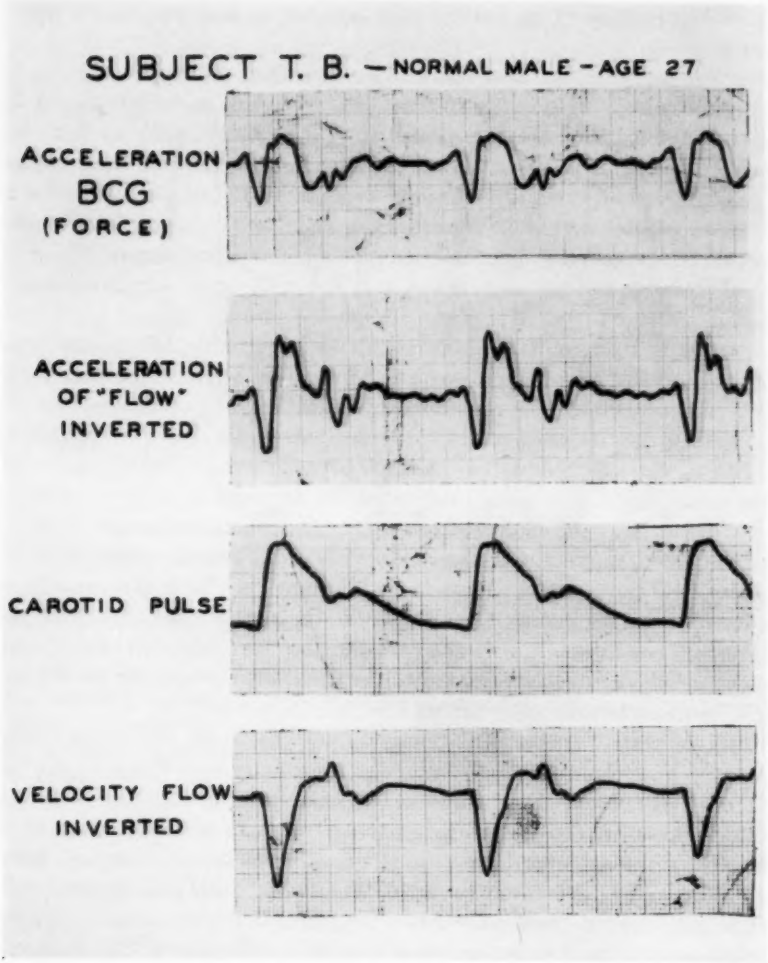

FIG. 5. By means of a Fry computer, the carotid pulse has been converted to a function approximating carotid flow. The resulting "flow" trace (not shown) has been differentiated once (lowest curve) and twice (second trace from top) to obtain the acceleration of "flow." When this record is inverted it exhibits a rather striking resemblance to the acceleration trace of the low-frequency BCG, and especially to the H-I-J sequence.

The carotid pulse reflects left ventricular forces and only those which are concerned with the movement of blood. It is hoped that comparison of the two top curves in a large number of subjects will aid in the separation of the right and left ventricular portions of the BCG, and also in the distinction of the deflections which are related to movements of the heart from those due to motion of blood.

Two of our present cardiovascular fellows, Drs. Cecil Coghlan and Gustavo Prieto of Chile, have recently recorded pressure pulse velocities in normal dogs. Their findings up to the present time are illustrated in Figures 6, 7, and 8, and summarized in Table I. The observations that the contraction of the left ventricle usually precedes that of the right, and that the mitral valve tends to close first, are in keeping with the findings of others in human subjects.

Indirect evidence of papillary muscle contraction as a very early systolic phenomenon was obtained in about three fourths of the experiments (Figs. 6 and 8; Table I). Perhaps the technique used was not sufficiently delicate to detect this event in the remaining animals.

About .05 second after the onset of excitation an interesting finding was encountered in each of ten dogs. The rate of pressure rise suddenly declined in the right ventricle and increased in the left (Figs. 7 and 8). This can be best explained by movement of the interventricular septum toward the left ven-

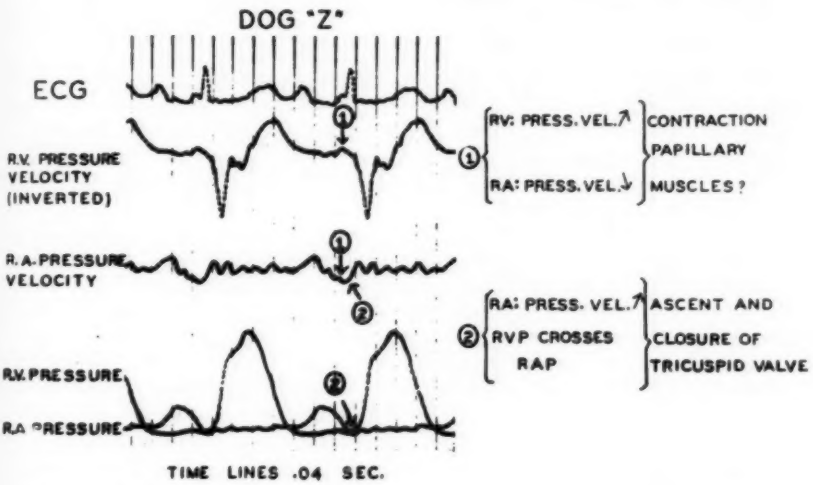

FIG. 6. The right ventricular derivative is inverted, a downstroke indicating an increase in the velocity of pressure rise.

The downward deflection in the atrial velocity curve (1) starting as the ventricular pressure rises is so small as to be of dubious significance in itself. The observation that this small motion is seen at this time in most complexes of most dogs makes it probable that it is a physiologic phenomenon rather than an artifact. Contraction of the papillary muscles, with descent of the atrioventricular cusps, is the logical explanation for simultaneous respective decline and rise in the atrial and ventricular curves.

A little later the atrial velocity record shows a sharp upstroke (2) which is ascribed to ascent of the tricuspid leaflets as the pressure continues to rise in the ventricle. About one hundredth of a second after this the valves close, as shown by crossing of the absolute pressure curves, and the velocity of ventricular pressure rise then becomes more abrupt.

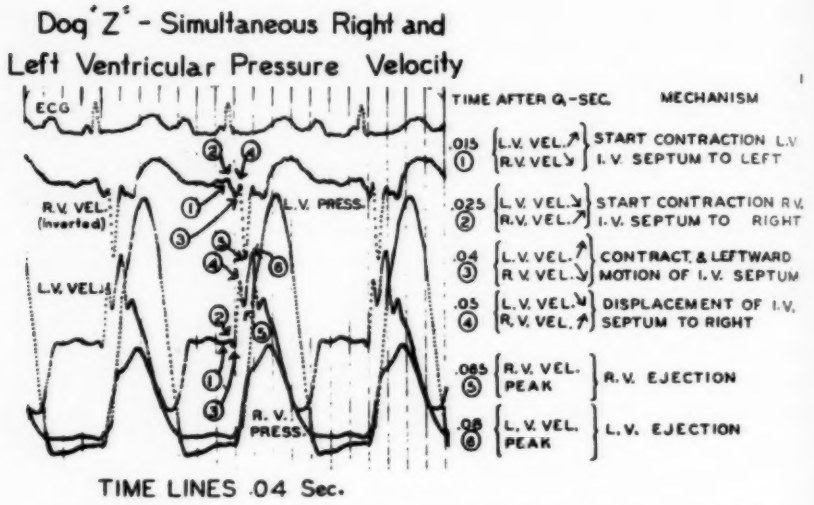
Dog "Z" - Simultaneous Right and
Left Ventricular Pressure Velocity
ECG
R.V. VEL. (inverted)
L.V. VEL.
L.V. PRESS.
R.V. PRESS.
TIME LINES .04 Sec.
TIME AFTER Q-SEC.
MECHANISM
.015 ① L.V. VEL.↗ R.V. VEL.↘ START CONTRACTION L.V. I.V. SEPTUM TO LEFT
.025 ② L.V. VEL.↘ R.V. VEL.↗ START CONTRACTION R.V. I.V. SEPTUM TO RIGHT
.04 ③ L.V. VEL.↑ R.V. VEL.↓ CONTRACT. & LEFTWARD MOTION OF I.V. SEPTUM
.05 ④ L.V. VEL.↘ R.V. VEL.↑ DISPLACEMENT OF I.V. SEPTUM TO RIGHT
.085 ⑤ R.V. VEL. PEAK R.V. EJECTION
.08 ⑥ L.V. VEL. PEAK L.V. EJECTION

FIG. 7. The velocity curve of the right ventricle is again inverted, an increasing rate of pressure rise being indicated by a decline in the record. The other traces are not inverted.

The first pressure rise is seen in the left velocity trace (1) and is associated with a small decline in the right velocity record. Shortly thereafter, the reverse occurs as the pressure starts to rise on the right (2). Presumably, each ventricle is producing a slight pull on the septum as it enters into contraction.

As the steep rise begins in the left ventricle, there is a momentary decrease in the rate of rise of right ventricular pressure (3). This is ascribed to leftward movement of the interventricular septum as it starts to contract. The steepest velocity of pressure rise in the right ventricle (4) is associated with a decline in the velocity of rise in the left chamber. This is attributed to rightward displacement of the septum by the higher pressure in the left ventricle.

The peak of the velocity trace indicates ejection is reached slightly sooner on the right (5) than on the left side (6).

tricle. During diastole this structure is not straight but somewhat rounded and bulges rightward. Contraction and hence shortening of the septum should cause a leftward-backward motion toward the left ventricle. Thus the paradox of a sudden decrease in the rate of rise of right ventricular pressure during early contraction may be explained.

Some years ago Dr. Isaac Starr reported a surprising observation. He found that destruction of the entire free wall of the right ventricle was well tolerated by dogs. Perhaps the reason is as follows: Eight of our ten dogs exhibited evidence of displacement of the septum to the right about .015 second before ejection began. At that time the velocity of pressure rise in the left ventricle suddenly diminished while that in the right increased (Figs. 7 and 8). The extent to which the left chamber may supply the energy for ejection by the right would seem to merit investigation.

The dog's aortic pressure record frequently displays a small decrease, beginning about .03 second before ejection. The decline is more pronounced in the aortic velocity records (Fig. 8D), which often exhibit an abrupt drop at a time when the left ventricular pressure is rising sharply. This suggests that the muscles at the base, which are attached to the aortic annulus, are exerting a downward pull. Evidence that such a pull exists in the normal human subject is shown in Figure 9. Tracings from the suprasternal notch displayed in each

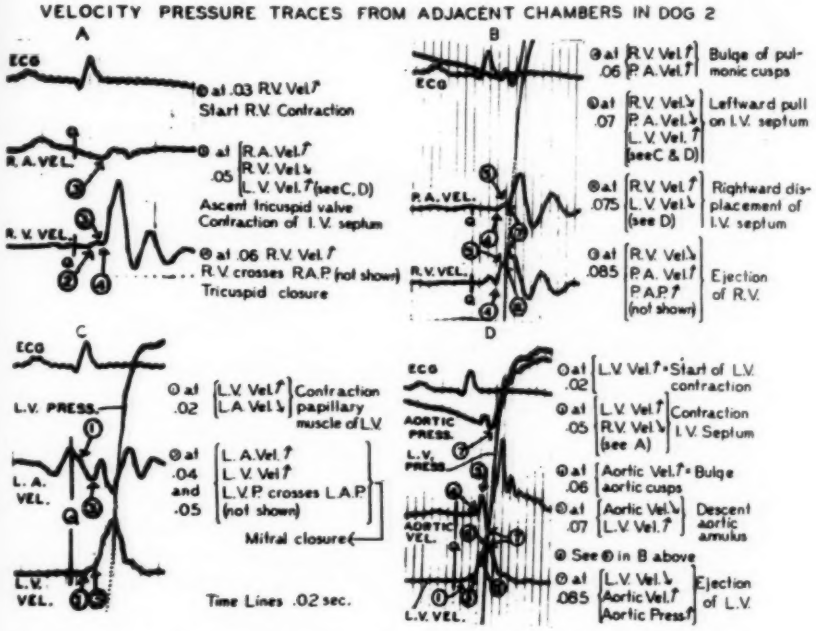
VELOCITY PRESSURE TRACES FROM ADJACENT CHAMBERS IN DOG 2
A
ECG
@ at .03 R.V. Vel.↑
Start R.V. Contraction
R.A. VEL.
@ at .05 R.A. Vel.↑ R.V. Vel.↓ L.V. Vel.↑ (see C, D)
Ascent tricuspid valve
Contraction of I.V. septum
R.V. VEL.
@ at .06 R.V. Vel.↑
R.V. crosses R.A.P. (not shown)
Tricuspid closure
B
ECG
@ at .06 R.V. Vel.↑ P.A. Vel.↑ Bulge of pulmonic cusps
@ at .07 R.V. Vel.↓ P.A. Vel.↓ L.V. Vel.↑ (see C & D) Leftward pull on I.V. septum
P.A. VEL.
@ at .075 R.V. Vel.↑ L.V. Vel.↓ (see D) Rightward displacement of I.V. septum
R.V. VEL.
@ at .085 R.V. Vel.↓ P.A. Vel.↑ P.A.P.↑ (not shown) Ejection of R.V.
C
ECG
@ at .02 L.V. Vel.↑ L.A. Vel.↓ Contraction papillary muscle of L.V.
L.V. PRESS.
@ at .04 and .05 L.A. Vel.↑ L.V. Vel.↑ L.V.P. crosses L.A.P. (not shown)
Mitral closure
L.A. VEL.
L.V. VEL.
Time Lines .02 sec.
D
ECG
@ at .02 L.V. Vel.↑ Start of L.V. contraction
@ at .05 L.V. Vel.↑ R.V. Vel.↓ (see A) Contraction I.V. Septum
AORTIC PRESS.
L.V. PRESS.
@ at .06 Aortic Vel.↑ aortic cusps Bulge
@ at .07 Aortic Vel.↓ L.V. Vel.↑ Descent aortic annulus
AORTIC VEL.
@ See @ in B above
@ at .085 L.V. Vel.↓ Aortic Vel.↑ Aortic Press.↑ Ejection of L.V.
L.V. VEL.

FIG. 8. The onset of pressure rise is earlier in the left ventricle and is associated with a sharp decline (papillary muscle contraction) in the left atrial velocity trace [C (1)]. No evidence of papillary muscle contraction is seen on the right, A, where contraction starts about .01 second later [A (2)].

The atrioventricular cusps appear to ascend, as shown by a rise in the atrial velocity trace a little earlier on the left [C (3)] than on the right [A (3)]. The decline in the rate of pressure rise in the right ventricle A (3), which is associated with a steeper rise on the left velocity trace [C (3)] is ascribed to contraction and leftward motion of the interventricular septum.

The pulmonary and aortic velocity traces show small upstrokes [B (4), D (4)] at .06 second after Q. These are ascribed to bulging of the semilunar cusps by the rising ventricular pressures. The aortic velocity record then exhibits a sharp dip [D (5)], which is presumably due to descent of the aortic ring.

The two ventricles eject together. At this time the peak velocities of ventricular rise are reached and the large increases in pressure velocity begin in the pulmonary and aortic traces [B (7), D (7)]. Just before this the septum is apparently pushed to the right because the velocity of pressure rise decreases in the left ventricle [D (6)] and increases in the right [B (6)].

TABLE I

Some Pressure Velocity Relationships During Pre-ejection Phase of Ventricular Systole in Dogs

Interpretation	Contraction of Papillary Muscles		Contraction and Leftward-backward movement of I. V. Septem	Displacement of I. V. Septum to right	Onset Contraction		Ascent A-V Valves	
	Left Ventricle	Right Ventricle			L.V.	R.V.	Mitral	Tricuspid
Event studied	L.V. Vel.↗ L.A. Vel.↘	R.V. Vel.↗ R.A. Vel.↘	R.V. Vel.↘ L.V. Vel.↗	R.V. Vel.↗ L.V. Vel.↘	Start Vel.↗	Start Vel.↗	L.A. Vel.↗	R.A. Vel.↗
Number of animals studied	6	7	8	8	10	8	6	6
Phenomenon observed in	4	5	All	6	All	All	All	All
Time after start of QRS, sec.								
Range	.02–.03	.015–.03	.04–.06	.05–.08	.015–.035	.015–.04	.03–.05	.025–.055
Average	.022	.024	.048	.062 (.017 before R.V. ejection)	.024	.028	.035	.038

instance a small footward movement occurring shortly before the headward motion produced by expansion of the aortic arch as ejection began. Apparently, the fibers at the base of the left ventricle begin to contract somewhat later than the subendocardial, septal, and right ventricular muscle bundles.

When one attempts to study the changes in pressure velocity in the human subject certain problems arise. Lags and artifacts may distort the records obtained by catheterization, which in any case usually yield data from the right chambers only. Curves from all chambers cannot usually be obtained at operation. They represent abnormal hearts and are beset by technical artifacts. Until these difficulties can be overcome the pressure velocity data from the human subject will have limited value. Nevertheless, such data as have been

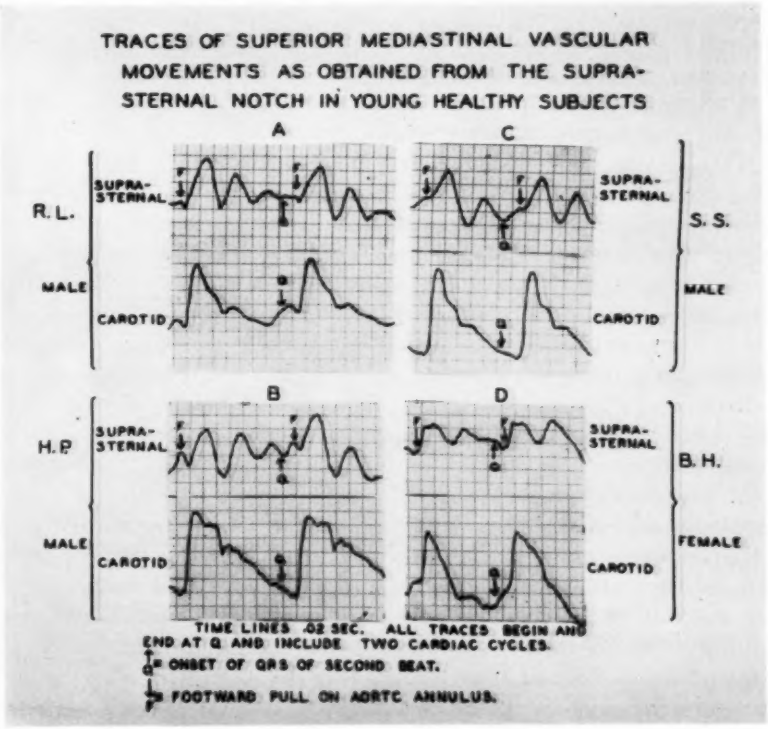

FIG. 9. A small inward motion in the suprasternal notch (↓F) is seen in each of the four subjects about .03 second before the start of the carotid upstroke. This movement corresponds well with the time of the sharp decline in the aortic velocity trace, as seen in Figures 4 and 8. It is ascribed to a footward pull on the aortic annulus by the muscles at the base of the left ventricle.

TABLE II

*Summary of Operating Room Observations on Pressure Velocity During Early Systole in Human Subjects**

	Simultaneous L.A. ↘ & L.V. ↗	Simultaneous R.V. ↘ & L.V. ↗	Simultaneous R.V. ↗ & L.V. ↘	Onset Pressure Velocity Rise		
				R.V.	L.V.	L.A.
Number of observations	12	5	5	6	14	13
Present in	6	4	5	6	14	13
Time after Q, sec.						
Average	.029	.056	.070	.050	.033	.047
Range	.015–.05	.045–.08	.055–.08	.02–.07	.02–.045	.025–.06
Probable mechanism	Contraction papillary muscles of L.V.	Contraction I.V. septum (or L.V.)	Displacement of I.V. Septum to rt.	Earlier excitation of L.V.		Ascent Mitral Cusps

* Various types of rheumatic and congenital lesions.

obtained would appear to be in general agreement with the findings in the dogs (Table II).

We may now return to the original objective of these studies—the attempt to understand the genesis of the normal precordial movements, and particularly in relation to the sequence of contraction. Aside from the initial small forward motion seen after isolated P waves, which often starts before ventricular excitation and which is clearly of atrial origin, four deflections may be observed prior to ejection. The first of these is small, inconstant, and appears to be related to the onset of pressure rise in the two ventricles as the subendocardial and papillary muscles contract. When present it starts about .03 second after Q and is characterized by an inward deflection in the right parasternal region. At the same time there is often outward motion (less commonly inward) in the left parasternal and apical regions (Figs. 1 and 10; Table III). These movements are tentatively ascribed to descent of the atrioventricular valves as the papillary muscles contract, because in the velocity pressure curves they correspond to atrial decline and to the start of the ventricular rise.

At about .05 to .07 second after the onset of excitation, two different movements occur. One of these is a backward deflection which is normally best seen in the left precordial and apical regions (Fig. 10, Table III). This motion is often exaggerated in both parasternal regions in patients with left ventricular hypertrophy and is diminished when the right ventricle is enlarged. It is commonly absent in patients with electrocardiographic evidence of septal infarction. This deflection appears to correspond to the simultaneous increase and decrease in the velocity of pressure rise in the left and right ventricles, respectively, which has been observed in dogs and in patients. It is ascribed

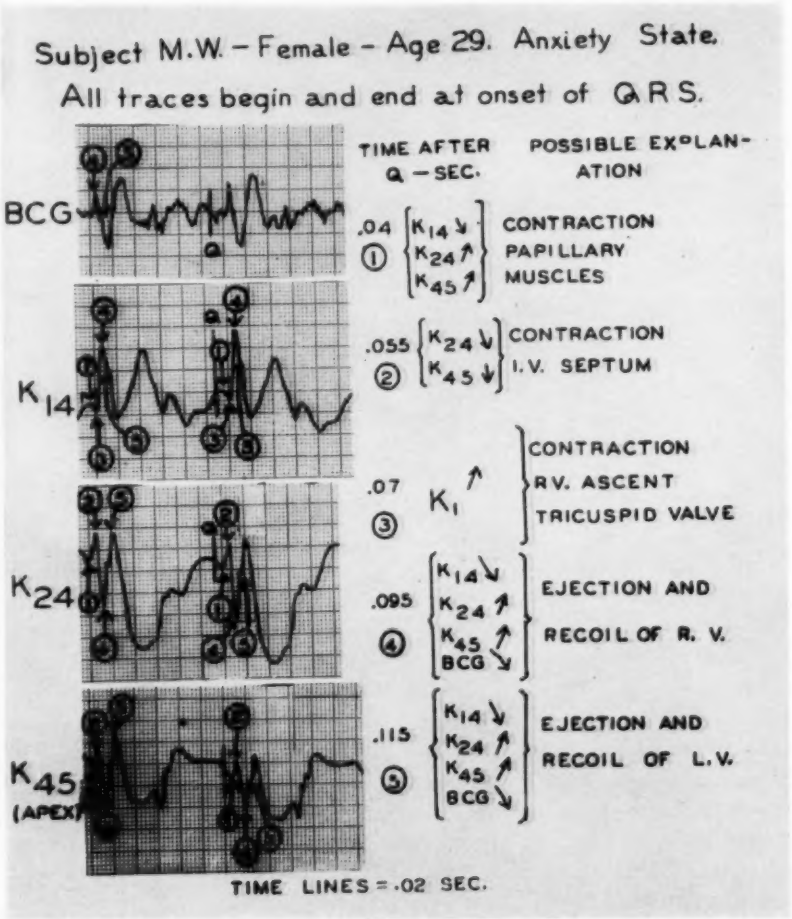
Subject M.W. – Female – Age 29. Anxiety State.
All traces begin and end at onset of QRS.
BCG
K14
K24
K45 (APEX)
TIME AFTER Q – SEC.
POSSIBLE EXPLANATION
.04 ① K14↘ K24↗ K45↗ CONTRACTION PAPILLARY MUSCLES
.055 ② K24↘ K45↓ CONTRACTION I.V. SEPTUM
.07 ③ K1↑ CONTRACTION RV. ASCENT TRICUSPID VALVE
.095 ④ K14↘ K24↗ K45↗ BCG↘ EJECTION AND RECOIL OF R. V.
.115 ⑤ K14↘ K24↗ K45↗ BCG↘ EJECTION AND RECOIL OF L.V.
TIME LINES = .02 SEC.

FIG. 10. The BCG is the acceleration trace of a low-frequency bed. K_{14} and K_{24} mean 4th interspaces in right and left parasternal lines, respectively. K_{45} means fifth interspace in the left midclavicular line.

In the K_{24} and K_{45} traces the arrow (5) should point at the beginning of the second part of the biphasic upstroke. This and the preceding arrow (4) refer to recoil as the two ventricles eject.

All of the motions discussed in the text are illustrated except those associated with the downward pull on the aortic annulus. This is not ordinarily seen in the usual precordial traces.

The forward movement at K_{14} due to atrial relaxation starts in this subject just before Q, and hence would not be confused with ventricular motions. The deflections of ventricular origin tend to be more abrupt than those related to atrial activity.

TABLE III

Summary of Precordial Movements During Pre-ejection Phase of Ventricular Systole

Movement	Best Seen at*	Time After Q, sec.	Effect of Ventricular Hypertrophy		Corresponding Change in Pressure Velocity	Remarks	Probable Mechanism
			Right	Left			
Forward	K_1	.03 before to .02 after	—	—	Atrium↘	Often starts before Q. In A-V block follows isolated P waves	Atrial Relaxation
Backward	K_1	.03 ± .01	—	—	Vent.↗ Atrium↘	Variable motions	Contraction papillary muscles
Backward	K_2 and K_X	.055 ± .015	Decrease	Increase	L.V.↗ R.V.↘	K_2 and K_X Often absent with septal infarction	Contraction of I.V. septum or L.V.
Forward	K_1	.04-.07	Increase	Decrease	R.A.↗	Marked in K_2 region, also in R.V.H.*	Contraction R.V. with (1) ascent tricuspid or (2) rightward rotation, or both
Footward	Suprasternal notch	.07-.10	Not yet studied		Aorta↘ L.V.↗	—	Contraction basal L.V.; footward pull on aortic ring

* K_1 = right parasternal: K_2 = left parasternal; K_X = apex. L.V. = left ventricle; R.V. = right ventricle; R.V.H. = ventricular hypertrophy

to a leftward and backward motion of the interventricular septum caused either by its own contraction or by the pull of the left ventricle.

The other motion, which starts about .06 second after the beginning of the QRS, is a forward deflection. In normal persons it is likely to be most marked in the right parasternal area (Fig. 10, Table III). When there is right ventricular hypertrophy this deflection is exaggerated and may be more pronounced in the left than in the right precordial region. This is the precordial lift which can be readily felt in most persons with right ventricular hypertrophy. The movement is often decreased or absent when the left ventricle is enlarged. The time of its occurrence corresponds well to a sudden rise in the pressure velocity curve of the right atrium of dogs and to the tricuspid component of the first heart sound in the human subject. These various findings lead to the conclusion that this forward motion is related to right ventricular activity. We do not yet know whether it is caused by the ascent of the tricuspid leaflets as closure occurs or by the "peculiar sidewise turning toward the right ventricle" which was first described by William Harvey.

The fourth ventricular motion prior to ejection is not usually seen in precordial traces but is readily apparent when records are taken from the suprasternal notch. This movement, which begins about .08 second after the start of excitation, appears to be a footward pull on the aortic ring by the basilar muscles of the left ventricle (Fig. 9, Table III).

The remaining precordial motions during early systole appear to be related to the onset of ejection and consist of sharp inward and outward deflections in the right and left precordial regions, respectively. The probable importance of volume change and of recoil in producing them has already been mentioned.

The investigations which have been described are compatible with the idea that the sequence of ventricular contraction is rather closely related to that of excitation. It would appear that the process begins in the subendocardial and papillary fibers, and starts slightly earlier on the left than on the right. The next areas appear to be the septum and the free wall of the right ventricle. The fibers at the apex appear to contract before those at the base. Presumably, (and here we have no direct evidence) the subepicardial fibers at the thick base of the left ventricle are relatively late in reaching the peak of their contraction.

These concepts of the sequence of contraction and of heart motion, which stemmed originally from studies of precordial movement, have been supported and extended by the observations of the rate of pressure change in chambers of the heart. Of particular interest is the observation that simultaneous changes in opposite directions in the pressure velocity of adjacent chambers appear to represent a guide to the movements of the intervening membrane. Such motions in turn furnish some idea of the functioning of the different portions which comprise the complex muscle mass of the heart. But this is again indirect

evidence. A more immediate approach to the study of these movements is being attempted and final concepts must await the verdict of these efforts.

I have not attempted to cite in detail the bedside applications of these studies of heart motion. As a by-product we have learned to detect right and left ventricular hypertrophy by simple palpation with a fair degree of accuracy. To say that one's hands are in most instances more reliable in this respect than the x-ray or electrocardiogram is to offer our fingers faint praise indeed. It is frequently possible to detect a myocardial infarction by palpation and occasionally at a time when the electrocardiogram is normal or borderline. Less frequently, the diagnosis of angina pectoris can be suspected when one feels a temporary localized bulge in a patient with atypical pain. These matters are mentioned only because they would have pleased Dr. Thayer, with his abiding interest in physical diagnosis.

As a final tribute to Dr. Thayer, I would like to illustrate his influence as a teacher by a personal instance. His demonstrations to the second-year class were filled with historical citations. His graphic descriptions of the accomplishments of Auenbrugger, Corvisart, Austin Flint and other giants not only aroused interest in medical history but led his students to hope that they too might make lasting contributions to our profession. It was soon clear that in Dr. Thayer's Valhalla, the chair at the head of the table was reserved for Laennec. Rarely did an hour pass without some mention of the father of auscultation. Each time, Dr. Thayer's eyes would become moist, and on more than one occasion he reached into the pocket of his long-tailed coat for the handkerchief needed to catch the tear of adulation. The realization that a man of his stature could feel such deep devotion and reverence for a medical Titan of the past was, for at least one student, the most inspiring formal educational experience of a lifetime. Thus, once again, I thank you for the chance to join with you in honoring the memory of a great teacher and a noble physician.

GENETIC FACTORS IN PREDISPOSITION TO PERNICIOUS ANEMIA[1]

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The first descriptions of pernicious anemia are ascribed to Combe in 1824 and to Addison in 1843, but it was not until the publication of the latter's famous treatise in 1855 that the clinical manifestations of the disorder became widely known (43, 55). The possibility that hereditary factors may be of importance in this disease was suggested in a case report by Howard in 1876 (42). By the end of the 19th century there were four additional reports (9, 54, 67, 89) which indicated that either "constitutional predisposition" or inheritance played a role. Subsequently the familial occurrence of pernicious anemia was described by many authors. In his review of this subject published in 1940, Askey (1) collected data from 235 families in which multiple cases had appeared. At least 113 additional families have been described (2, 14, 20, 22, 27, 32, 40, 63, 65, 75, 79, 81, 83, 86, 87, 90). Pernicious anemia has been encountered in both members of pairs of identical twins, an observation which strengthens the evidence for hereditary factors in the disease (1a, 2, 21, 30, 32, 40, 53, 65, 86).

The description of gastric anacidity in a patient with pernicious anemia (12) and the subsequent discovery that this condition almost always accompanies the disease, in fact preceding it by years in some instances (1, 18, 28, 91), led several investigators to test the gastric secretions of relatives of patients with pernicious anemia. With the sole exception of the study by Neuburger (73), these reports have shown a significant increase in the number of achlorhydric individuals in these families as compared with control groups (1, 14, 17, 23, 53, 65, 96).

Carcinoma of the stomach is known to be more common in patients with pernicious anemia than in persons of the same age in the general population (4, 51, 52). This neoplasm occurs with similarly increased frequency in relatives of patients with pernicious anemia (65). Several families have been described

[1] This work was carried out under Contract AT(30-1)-1208 between the Atomic Energy Commission and The Johns Hopkins University.

[2] This study was initiated while Dr. McIntyre was a Fellow of the American Cancer Society, 1955–1956. Present address: The Confederate Memorial Medical Center, Shreveport, Louisiana.

in which one parent was known to have cancer of the stomach and two or more of the offspring developed pernicious anemia (1, 8, 46, 50, 53).

The frequency with which multiple cases of pernicious anemia are seen in single family groups, the coincidental occurrence of this disease in identical twins, and the increased frequency with which otherwise unaffected family members have been found to have achlorhydria or carcinoma of the stomach are together suggestive that there is a genetically determined predisposition to the development of the disease. In addition, certain constitutional and presumably inherited physical characteristics are known to be common in patients with pernicious anemia, for example, fair skin, premature greying of the hair, broad forehead, blue or light-colored eyes, wide interpupillary diameter, deep and short chest and wide subcostal angle (15, 24, 25, 48). Several studies have shown an increase in the incidence of blood group A in patients with pernicious anemia as compared with the general population (11, 41, 95).

Reports collected from the literature show great variety in the patterns with which pernicious anemia appears in family groups. In some the disease can be traced through direct lines for three generations (19, 37, 44, 58, 66, 69, 87, 92, 96) and in one family for four generations (56). Apparently unaffected members of a sibship with multiple cases of pernicious anemia have had offspring who developed the disease (33, 39, 61, 62). Parents who had no family history of the disorder, and who themselves were unaffected, have had two or more offspring with pernicious anemia (44, 45, 80, 81, 82, 83, 91, 93, 94). Where both parents have been known to have the disease, some, all, or none of the offspring have developed it (14, 56, 58, 59, 65).

Family studies depending upon clinical manifestations of pernicious anemia are not adequate to delineate the genetic basis of the disease. There is much to suggest that factors other than inheritance influence the appearance of clinical symptoms. A number of studies have shown that the incidence of pernicious anemia increases strikingly with age (26, 36, 61, 64, 85). When these observations are considered in terms of the population density in various age groups there is an essentially linear relationship between age and the frequency of the disease in adults (26, 61, 64). Family studies have been necessarily incomplete because of death of some members from unrelated causes before pernicious anemia was manifested by clinical symptoms. Other members, asymptomatic at the time of the study, have developed pernicious anemia at a later date. Many authors have described "pre-pernicious anemia" states manifested by achlorhydria, macrocytosis and sometimes glossitis in otherwise normal non-anemic family members (2, 10, 14, 61, 74, 77, 79, 85). The report of Faber and Gram (29) in 1924 illustrates the erroneously low number of family members considered to be affected in ordinary clinical studies. At the time of their original examination two of six siblings were found to have pernicious anemia. Nine years later Gram discovered that another sibling had developed the dis-

ease (35). Mosbech (65) in 1953 reported one other suspected case of pernicious anemia in this family. Similarly, a family reported by Meulengracht (61) in 1925 to have four siblings with pernicious anemia was re-examined by Mosbech in 1951 and a fifth sibling was then found to be affected (65).

Age is not the only factor influencing the time of appearance of the clinical manifestations and in certain families offspring have been reported as developing the disease as much as 17 years before its recognition in the parent (63, 68). The increase in the number of cases of pernicious anemia seen in Europe during and immediately after World War I suggests that economic and most likely dietary factors are of considerable importance (5, 85). The identical twins described by Kaufmann and Thiessen (53) are of interest in this regard. The poverty-stricken twin developed pernicious anemia seven years before her well-to-do sibling experienced the onset of symptoms of subacute combined degeneration of the spinal cord. Two studies (36, 85) have shown that a larger number of new cases of pernicious anemia appear during the late winter and spring months, again suggesting the possible affect of dietary or other factors. Infestation with *Diphyllobothrium latum* may precipitate pernicious anemia in susceptible persons years before they develop "spontaneous" pernicious anemia (7, 82). It seems possible that other infections or diseases could have a similar effect.

The clinical manifestations of pernicious anemia are wholly attributable to a deficiency of vitamin B_{12}. The basic lesion is a peculiar disorder of the gastric mucosa, which fails to secrete "intrinsic factor", a substance required for normal absorption of vitamin B_{12} from the gastrointestinal tract. The gastric lesion prevents the secretion of hydrochloric acid and gastric enzymes. This lesion also apparently predisposes to polyp formation and to neoplasia. A reasonable hypothesis is that the abnormality of the stomach develops as the result of genetically determined processes. Whether pernicious anemia thereafter appears probably depends upon the severity and duration of the gastric atrophy, the dietary intake of vitamin B_{12} and folic acid, and conditions which may alter the need for these vitamins.

A more direct approach to the study of genetic factors in pernicious anemia would be provided by examination of the gastric mucosa and its secretions. Assay of the gastric contents for intrinsic factor is possible but is too cumbersome a technique to be applicable to extensive family studies. On the other hand, ability to absorb vitamin B_{12} can be measured readily by using tracer amounts of the vitamin labelled with radioactive cobalt. The amount of orally administered vitamin which is absorbed is related to the amount of intrinsic factor which is available in the gastrointestinal tract. In patients with pernicious anemia, there is within certain limits a stoicheiometric relationship between the amount of vitamin B_{12} absorbed and the amount of intrinsic factor available (3). Intestinal disease and other disorders unrelated to deficiency of

intrinsic factor may limit the absorption of the vitamin; however, in an apparently healthy population it is a reasonable assumption that such disorders are not present. By the use of the tracer method in a preliminary study in this laboratory (60) asymptomatic members of the families of patients with pernicious anemia were compared with other normal persons not known to be related to patients with pernicious anemia. There was a difference between the two groups, a higher frequency of poor absorbers being found in the family members. Callender and Denborough (14) in a similar study demonstrated impaired absorption of vitamin B_{12} in a number of *achlorhydric* members of the families of patients with pernicious anemia. These investigators did not measure absorption of vitamin B_{12} in those family members who had free acid in the gastric secretion. The investigation to be described consists of the further application of the radioactive tracer method in an effort to determine the mode of inheritance of predisposition to pernicious anemia.

METHODS

Selection of family members

Patients with pernicious anemia were included in this study only if the diagnosis had been well documented. Most had been observed to have megaloblastic anemia and other typical features of the disease in relapse, with prompt and complete response to vitamin B_{12} or liver therapy. All had histamine-fast achlorhydria. Patients who had had gastrectomy and those with structural or functional abnormalities of the intestine were not included. In cases in which the diagnosis had not been completely established on clinical grounds, confirmation was provided by radioactive tracer studies. In these cases vitamin B_{12} labelled with cobalt^{60} was poorly absorbed from the gastrointestinal tract except when administered with intrinsic factor concentrate. The only other selective factors were the availability of relatives and their willingness to participate. Female patients were more cooperative in arranging for examination of their relatives. For this reason there is a disproportionate number of female probands. One hundred and six relatives of 34 patients were examined. Data pertaining to an additional family were discarded when blood group analysis cast doubt on the paternity of the offspring.

The relatives of the probands included 4 parents (1 father; 3 mothers), 23 sibs (12 sisters; 11 brothers), 55 children (25 sons; 30 daughters), 2 half-sibs (1 half-brother; 1 half-sister), 9 nephews, 2 nieces, 9 grandchildren (5 granddaughters; 4 grandsons), and 2 first cousins (maternal). Both sexes were equally represented among these relatives, there being 52 males and 54 females. Both white and negro patients were admitted to the study, the latter constituting 16 per cent of the group.

Selection of controls

Previous studies (60) have shown that the average value for absorption of radioactive vitamin B_{12} is somewhat lower in patients with various diseases unrelated to pernicious anemia than in healthy subjects. In only five of the 106 relatives was there any known disease. Therefore, data were collected from a similarly healthy group of 97 normal persons not known to be related to patients with pernicious anemia. These controls were selected on the basis of good health and a history negative for the familial occurrence of pernicious anemia or cancer of the stomach.

Because of availability, more males than females are included in the control group (62 males; 35 females). This difference arose from the fact that the controls were provided principally from two groups: persons connected with the Johns Hopkins Hospital (physicians, nurses, medical students), and volunteers from a biology class on the Homewood Campus of The Johns Hopkins University. The latter control group is further responsible for most of the difference in age as well as sex between controls and relatives of pernicious anemia probands. There were no negroes in the control group. The difference in age is set forth in general terms in Figure 3. The mean age of the total control group is 30.35 years, of the relatives 36.23 years. Divided into three age groups (up to 20 years, 21–40 years, and 41 years of age and above), the respective means of the control groups are 18.47 years, 27.18 years, and 53.05 years; those of the relatives 13.16 years, 31.24 years, and 54.07 years. Clearly, the relatives differ most from the controls in the youngest age group, where, unlike the controls, many young children are included. To a smaller extent, the controls are younger in the middle group because of the inclusion of a relatively large number of young college students; and finally, the smallest difference, one of only a year, occurs in the most elderly group. The bearings of these differences in sex, age and racial composition between the relatives and control group will be examined below, in the section of the paper dealing with Results.

Measurement of vitamin B_{12} absorption

A modification of the urinary excretion method of Schilling (84) was used to estimate the ability of subjects to absorb vitamin B_{12} labelled with cobalt^{60}. With this method approximately one-third of the radioactive vitamin B_{12} absorbed from the gastrointestinal tract is excreted in the urine (13). The oral dose of 0.5 μg. Co^{60}-vitamin B_{12}[2] (specific activity 0.2–0.8 microcurie/μg.) was diluted with distilled water to an etched mark (approximately 120 ml.) in a labelled beaker and the radioactivity measured. This material was administered

[2] All of the vitamin B_{12} used in these studies, including that labeled with Co^{60}, was generously provided by Merck & Company, Inc.

to the subject after a period of fast of at least five hours, additional water being used to insure that all of the radioactive material was ingested. After one hour, during which nothing was taken by mouth, an injection of 1000 μg. of crystalline vitamin B_{12} was given intravenously. Urine was collected for 25½ hours after the oral dose. This unusual time period was chosen for the convenience of the family members who performed the tubeless test for gastric acidity on the morning following the institution of the Schilling test. Since little vitamin B_{12} is excreted after the twelfth hour, the results of these tests are comparable to those in which the urine collection period was 24 hours (60).

In order to ensure accurate measurement of the proportion of administered radioactivity which appeared in the urine, the same beaker used for the oral dose was employed for urine radioactivity measurements. The beaker was first demonstrated to be free of residual radioactivity. In tests in which Co^{60}-vitamin B_{12} of low specific activity was used, the urine was concentrated by evaporation. Radioactivity was measured using a shielded scintillation crystal, the beaker being held in a constant and fixed position by a thin plastic dish. Results are expressed as the percentage of orally administered radioactivity excreted in the 25½ hour urine specimen.

The normal subjects and family members were ambulatory and not residing in the hospital. Since loss of any portion of the urine specimen would produce an erroneously low result, every effort was made to repeat the test in subjects who excreted less than 15.0 per cent of the administered dose. For the same reason, when more than one test was performed on a subject, the highest value obtained was assumed to represent the individual's ability to absorb vitamin B_{12}. At least two weeks were allowed to elapse between successive tests.

Hematologic studies

The hematocrit value and ABO blood types were determined on blood from many of the family members. Randomly selected blood bank donors served as controls for the study of blood group distribution. In some instances Rh, Kell and Duffy blood types were determined to verify paternity.

Test of gastric acidity

The Improved Diagnex® Azure A[4] test of tubeless gastric analysis was performed on 86 family members. The test, well described elsewhere (6, 88), was initiated on the morning after the Schilling test was begun. The initial 23½ hour urine specimen served as the control sample and the two-hour terminal urine collection was kept separately for estimation of dye excreted. This small sample was later added to the previous specimen for determination of radio-

[4] Improved Diagnex® was generously made available for this study by E. R. Squibb & Sons.

activity. Whenever possible, negative results were checked by gastric intubation and the administration of alcohol or histamine.

RESULTS

Absorption of vitamin B_{12}

Results of Schilling tests performed on the family members and normal controls are shown in Figure 1 and compared with those of other patient groups. Each of 51 patients with pernicious anemia excreted less than five per cent of the orally administered radioactive tracer. A group of patients with diseases unrelated to pernicious anemia showed wide variation in the test results. Forty-one per cent of these excreted 15.0 per cent or less. The normal control group appears distinctly different from the patient groups, the majority of subjects having results between 15.0 per cent and 30.0 per cent with a roughly normal frequency distribution. The family group has a bimodal distribution, with one mode falling well within the range of the control group and the other well below (Fig. 2). Table I summarizes the results of these tests. Many more of the relatives had lower test results and fewer fell into the high normal range.

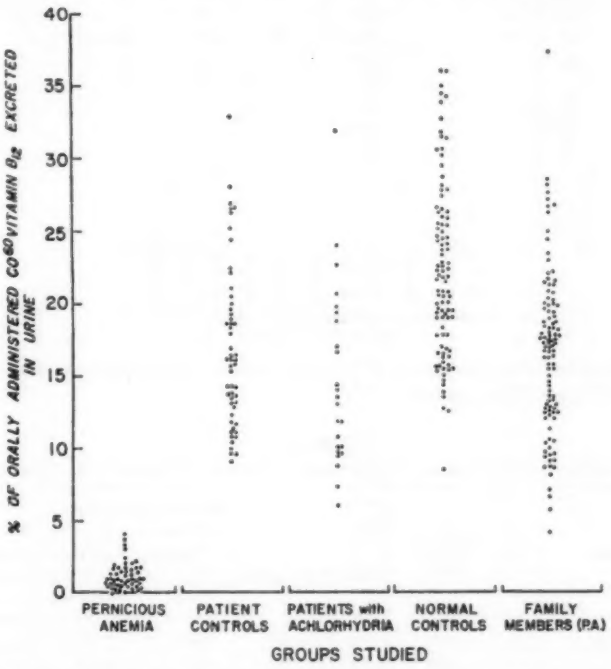

FIG. 1. Results of Schilling tests in relatives of patients with pernicious anemia compared with those of other normal subjects and of several patient groups.

Only 8 of the 97 control subjects excreted 15.0 per cent or less and only one less than 12.5 per cent. In contrast, 40 of the 106 relatives had excretion values of 15.0 per cent or less and 21 of these fell below 12.5 per cent. The Chi-square value for the difference between normal absorbers (above 15.0 per cent) and low absorbers (below 15.0 per cent) in the comparison between the relatives and controls is 5.7 for one degree of freedom, and is significant at the 2 per cent level. A detailed tabulation of the results of the tests in control and family groups is provided in Tables II and III. Excretion values of 15.0 per cent or below were confirmed by repeated testing in 20 of 40 family members and in 6 of 8 control subjects. Of the five relatives who were known to have some organic disease, four had test results above 17.0 per cent. The fifth is discussed below (Mother of M. L.).

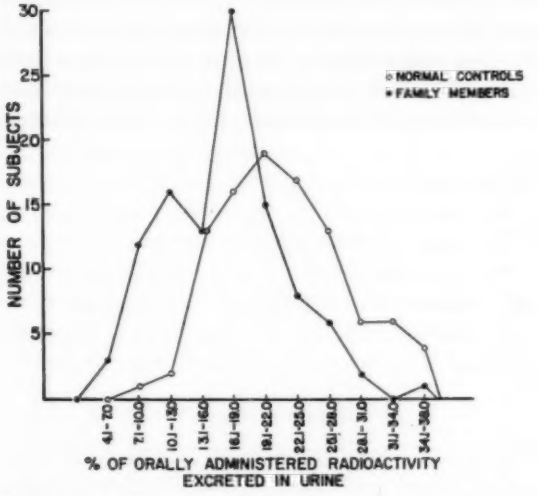

FIG. 2. Distribution of the results of Schilling tests in normal controls and in relatives of patients with pernicious anemia.

TABLE I

Results of Schilling Tests in Normal Controls and in Relatives of Patients with Pernicious Anemia

Group	Number of Subjects Tested	Results of Schilling Tests			
		Range of test results	Number of subjects with test result		
			>15%	12.5-15.0%	<12.5%
Normal controls	97	8.4-36.0%	89	7 (5)*	1 (1)
Relatives	106	4.2-37.5%	66	19 (5)	21 (15)

* Numbers in parentheses indicate the number of subjects in each group whose abnormal or borderline test results were confirmed by repeated tests.

TABLE II

Normal Control Subjects

Subject	Age	Race and Sex	SCHILLING TEST	
			Radioactivity excreted in urine (as per cent of orally administered radioactivity)	
			1st Test	2nd Test
1. E. G.	22	WF	28.9	
2. A. B.	29	WF	36.0	
3. T. G.	41	WF	21.6	
4. A. B.	26	WM	26.5	
5. E. M.	26	WM	27.9	26.5
6. A. F.	25	WF	24.5	
7. A. M.	26	WM	36.0	
8. L. G.	23	WM	17.6	
9. H. K.	28	WM	31.5	
10. J. A.	26	WM	22.5	
11. W. N.	26	WM	13.9	20.8
12. O. F.	27	WM	14.1	17.0
13. H. P.	20	WF	24.9	
14. S. S.	25	WF	34.5	
15. G. S.	27	WM	26.1	
16. J. S.	26	WM	28.2	
17. E. K.	23	WM	21.6	
18. M. S.	24	WM	14.5	31.6
19. V. S.	24	WM	22.8	
20. D. M.	23	WM	11.2	12.6
21. A. M.	24	WM	13.4	
22. J. G.	26	WM	8.4	4.7
23. E. B.	24	WM	16.5	
24. K. C.	26	WF	17.9	
25. R. W.	44	WF	19.0	
26. E. S.	23	WF	19.4	
27. A. E.	17	WF	22.3	
28. A. N.	46	WF	15.9	
29. W. S.	29	WM	16.6	
30. J. F.	24	WM	12.5	10.5
31. J. C.	23	WM	19.0	
32. S. E.	24	WM	16.3	
33. C. H.	23	WF	14.7	
34. M. F.	43	WF	20.8	
35. E. K.	51	WF	13.5	26.3
36. P. K.	37	WF	18.2	
37. R. R.	17	WM	12.8	30.6
38. S. K.	18	WM	11.5	17.7
39. P. G.	19	WM	11.0	15.3
40. G. K.	19	WM	12.2	20.5
41. E. F.	24	WM	15.4	
42. J. S.	26	WM	19.3	
43. G. S.	24	WM	8.4	15.0
44. R. S.	18	WM	26.4	
45. R. B.	17	WM	12.2	24.5
46. J. C.	19	WM	21.6	
47. D. P.	28	WM	25.9	
48. J. B.	18	WM	33.9	
49. D. M.	19	WM	29.6	

TABLE II—*Continued*

Subject	Age	Race and Sex	SCHILLING TEST	
			Radioactivity excreted in urine (as per cent of orally administered radioactivity)	
			1st Test	2nd Test
50. C. D.	22	WM	27.8	
51. R. B.	18	WM	24.2	
52. J. L.	19	WM	34.0	
53. J. R.	18	WM	19.8	
54. H. Y.	17	WM	26.5	
55. T. P.	18	WM	27.4	
56. J. P.	20	WM	30.2	
57. R. E.	19	WM	22.4	
58. S. S.	19	WM	20.3	
59. M. K.	18	WM	15.4	
60. T. R.	19	WM	35.0	
61. J. M.	19	WM	22.3	
62. R. G.	18	WM	25.3	
63. D. L.	19	WM	19.0	
64. B. M.	19	WM	31.3	
65. A. E.	19	WM	15.4	
66. J. M.	18	WM	20.6	
67. N. W.	19	WM	21.7	
68. M. L.	19	WM	14.1	22.8
69. R. H.	18	WM	16.5	
70. B. G.	51	WM	16.7	
71. A. G.	30	WM	19.0	
72. M. L.	21	WM	23.6	
73. H. F.	45	WF	23.8	
74. F. B.	48	WM	21.8	
75. C. T.	40	WF	24.4	
76. H. S.	46	WF	30.6	
77. R. R.	57	WF	24.0	
78. E. L.	57	WF	25.4	
79. R. W.	58	WF	24.7	
80. H. L.	59	WF	20.0	
81. S. S.	44	WF	22.1	
82. T. L.	63	WF	19.5	
83. B. T.	39	WF	15.2	
84. H. B.	25	WF	19.0	
85. A. L.	59	WF	32.8	
86. M. E.	63	WF	15.5	
87. T. R.	38	WF	19.6	
88. J. U.	74	WF	16.7	
89. P. H.	24	WF	23.3	
90. F. F.	38	WF	20.0	
91. C. E.	45	WM	15.7	
92. B. W.	35	WM	19.5	
93. E. L.	65	WF	25.1	
94. L. C.	38	WF	16.2	20.3
95. A. C.	32	WM	12.3	13.9
96. C. C.	43	WM	13.9	14.5
97. A. C.	65	WF	25.7	

TABLE III

Relatives of Patients with Pernicious Anemia

Proband		Age	Race and Sex	Relationship to Proband	Schilling Test: Radioactivity excreted in urine (as per cent of orally administered radioactivity)			Gastric Acidity		
								Azure A test	Gastric analysis	
					1st Test	2nd Test	3rd Test		With histamine	With alcohol
S. B.	1	35	CF	daughter	14.0					
A. B.	2	56	WF	daughter	12.6			+		
B. D.	3	48	CF	sister	12.4			−		−
M. D.	4	40	WM	son	2.4 (7–55)	8.1 (9–55)	6.6 (1–57)	+	+	
L. E.	5	49	WF	daughter	18.4			+		
I. F.	6	45	WM	son	16.7					
V. F.	7	54	CM	brother	9.1					
V. F.	8	65	CM	brother	17.8					
V. F.	9	63	CF	sister	7.1 (7–56)	9.6 (4–57)		+		
S. H.	10	35	WM	son	10.8 (6–56)	13.0 (10–56)		−	−	
S. H.	11	29	WM	son	18.1			+		
S. H.	12	32	WF	daughter	27.2			+		
S. H.	13	36	WF	daughter	20.0			+		
S. H.	14	25	WM	son	22.6			+		
S. H.	15	47	WM	half-brother	9.2 (7–56)	7.7 (4–57)		+		
S. H.	16	49	WF	half-sister	14.8			+		
S. H.	17	67	WF	sister	17.0					
S. H.	18	60	WF	sister	13.5			−		
S. H.	19	65	WM	brother	9.5 (8–56)	6.4 (11–56)		−		
S. H.	20	14	WM	nephew	19.9			+		
S. H.	21	11	WM	nephew	22.7			+		
S. H.	22	19	WM	nephew	22.0			+		
S. H.	23	13	WM	nephew	18.7			+		
S. H.	24	14	WF	grand-daughter	14.5			+		
L. H.	25	45	CM	son	17.3 (5–55)		10.4 (6–55)			
M. I.	26	41	WF	daughter	12.8 (8–56)	9.4 (3–57)		+		+
M. I.	27	51	WM	son	21.7			+		
M. I.	28	45	WM	son	23.0			+		
M. I.	29	10	WF	grand-daughter	18.0			+		
N.K.	30	37	CF	daughter	9.1 (12–56)	21.6 (4–57)		+		

TABLE III—*Continued*

Proband		Age	Race and Sex	Relationship to Proband	Schilling Test: Radioactivity excreted in urine (as per cent of orally administered radioactivity)			Gastric Acidity: Azure A test	Gastric Analysis	
					1st Test	2nd Test	3rd Test		With histamine	With alcohol
N.K.	31	16	CM	grandson	17.9			+		
E.K.	32	14	WM	son	25.0			+		
E.K.	33	12	WM	son	17.6			+		
E.K.	34	8	WM	son	26.8			+		
C.L.	35	30	WF	daughter	20.9			+		
C.L.	36	33	WF	daughter	13.7			+		
M.L.	37	86	WF	mother	3.3	5.7				
					(12–56)	(1–57)		+		
M.L.	38	61	WF	sister	10.9	12.6				
					(12–56)	(1–57)		+		
M.L.	39	53	WM	brother	2.6	8.8				
					(12–56)	(1–57)		+		
T.L.	40	33	WF	daughter	17.8			+		
T.L.	41	62	WF	sister	15.9	12.0				
					(3–57)	(5–57)		+		
T.L.	42	49	WM	brother	15.5	13.2				
					(3–57)	(5–57)		+		
J.L.	43	38	WF	daughter	7.1	6.6				
					(2–56)	(5–56)		−	−	
J.L.	44	29	WM	son	8.8	7.7				
					(5–56)	(4–57)		−	−	
J.L.	45	37	WM	son	15.0					
J.L.	46	27	WM	son	17.3			+		+
J.L.	47	35	WM	son	18.8			+		
J.L.	48	24	WF	daughter	22.1					
O.M.	49	44	WM	son	12.6	14.4				
					(1–57)	(4–57)		+		
H.M.	50	53	WF	sister	8.4	10.4				
					(10–56)	(4–57)		−		−
H.M.	51	56	WF	sister	18.4					
H.M.	52	50	WF	sister	16.2					
H.M.	53	52	WM	brother	6.5	6.6				
					(7–56)	(11–56)		+		
H.M.	54	55	WF	sister	6.1	20.0				
					(7–56)	(4–57)		+		
H.M.	55	23	WM	nephew	28.5			+		
H.M.	56	10	WM	nephew	8.7	17.8				
					(11–56)	(4–57)		+		
H.M.	57	22	WM	nephew	12.5			+		
F.M.	58	31	CM	son	12.8	5.0				
					(1–56)	(6–57)		+		
F.M.	59	32	CM	son	17.3	17.4				
					(1–56)	(6–57)		−		
F.M.	60	33	CF	daughter	16.2					

TABLE III—*Continued*

Proband		Age	Race and Sex	Relationship to Proband	Schilling Test			Gastric Acidity		
					Radioactivity excreted in urine (as per cent of orally administered radioactivity)			A test Azure	Gastric Analysis	
					1st Test	2nd Test	3rd Test		With histamine	With alcohol
J.Me.	61	64	WM	brother	11.3 (4–56)	16.8 (3–57)		+		
J.Me.	62	60	WM	brother	20.4					
J.M.	63	56	WF	daughter	17.4			+		
J.M.P.	64	11	CM	son	13.5 (10–56)	24.5 (3–57)		+		
J.M.P.	65	29	CF	sister	1.9 (10–56)	20.2 (3–57)		+		
A.M.	66	35	WF	daughter	22.2					
H.P.	67	30	WF	daughter	37.5 (4–55)	27.8 (5–55)				
H.P.	68	34	WM	son	26.9 (4–55)	24.8 (5–55)				
A.R.	69	53	CF	daughter	10.0 (7–55)	8.7 (11–55)	6.0 (9–57)	−	−	
A.R.	70	65	CM	brother	19.9					
M.S.	71	30	WM	son	4.2			−		
M.S.	72	39	WF	daughter	16.3			+		
M.S.	73	28	WF	daughter	12.9			−		
M.S.	74	44	WM	son	10.3 (1–57)	16.9 (3–57)		+		
M.S.	75	47	WF	daughter	10.0			+		
M.S.	76	26	WF	daughter	15.7			+		
M.S.	77	9	WM	grandson	17.3			+		
M.S.	78	14	WM	grandson	19.7			+		
E.S.	79	47	WF	sister	7.6 (2–57)	17.6 (3–57)		+		
E.S.	80	24	WM	son	9.9 (2–57)	11.4 (3–57)		+		
E.S.	81	33	WM	brother	5.3 (2–57)	16.8 (3–57)		+		
E.S.	82	8	WF	daughter	17.7			+		
E.S.	83	45	WM	brother	12.1 (2–57)	11.0 (2–57)		+		
E.S.	84	25	WF	niece	27.6			+		
E.S.	85	8	WM	nephew	11.1 (3–57)	19.4 (3–57)		+		
E.S.	86	15	WM	nephew	18.6			+		
E.S.	87	14	WF	niece	26.4			+		
E.S.	88	41	WM	maternal cousin	13.4					
E.S.	89	53	WF	maternal cousin	12.6					
H.S.	90	75	WF	mother	13.3					

TABLE III—*Continued*

Proband	Age	Race and Sex	Relationship to Proband	Schilling Test: Radioactivity excreted in urine (as per cent of orally administered radioactivity)			Gastric Acidity: Azure A test	Gastric Analysis	
				1st Test	2nd Test	3rd Test		With histamine	With alcohol
T.S. 91	27	WF	daughter	19.0			+		
T.S. 92	19	WF	daughter	11.5 (11–56)	12.1 (12–56)		+		
S.T. 93	38	CF	daughter	28.6			+		
S.T. 94	32	CM	son	10.6			−	−	
E.T. 95	14	WF	daughter	19.5			+		
E.T. 96	17	WF	daughter	19.1			+		
E.T. 97	20	WF	daughter	17.6			+		
F.W. 98	43	WF	daughter	6.7 (9–56)	10.0 (10–56)		+		
F.W. 99	12	WF	granddaughter	8.8 (9–56)	23.6 (10–56)		+		
F.W. 100	21	WF	granddaughter	6.2 (9–56)	9.6 (10–56)		+		
F.W. 101	9	WM	grandson	10.5					
F.W. 102	18	WF	granddaughter	12.7					
M.W. 103	40	WM	son	21.3			−		
M.W. 104	52	WF	daughter	18.4			−		
A.O,M. 105	48	WF	mother	26.2			−		
A.O,M. 106	61	WM	father	11.6 (3–57)	15.8 (3–57)		+		

No demonstrable difference in regard to absorption of vitamin B_{12} exists in respect to sex, in either the relatives or the controls. In the former, the percentage of low absorbers is 35.3 in the males, 41.5 in the females. Chi-square is 0.422 for one degree of freedom, and P lies between 0.50 and 0.70. In the control group, 7 of 62 males (11.3 per cent) and 1 of 35 females (2.9 per cent) are classified as low absorbers. The number of female control individuals is unfortunately small, a fact which leads to considerable uncertainty as to whether males might in general more frequently fall below the chosen limit for normal B_{12} absorption. However, if Yates' correction is applied to the data and a Chi-square is calculated, it is very low, 0.3365 for one degree of freedom and again P falls between 0.50 and 0.70. The absence of any significant sex difference in absorption in the relatives, and the fact that in the controls the males, but in the relatives the females, are somewhat more frequently low absorbers, make it unlikely that the greater representation of males in the controls than in the relatives or in a random sample of the general population exerts any significant bias affecting the comparisons of interest.

In regard to the inclusion of a few negro individuals among the probands

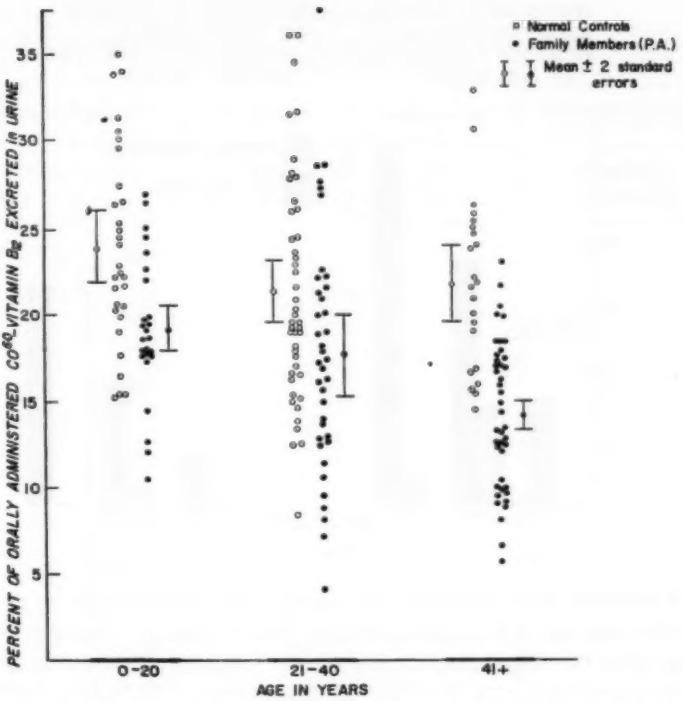

FIG. 3. Results of Schilling tests in normal controls and relatives of patients with pernicious anemia in relation to age.

and relatives, even less indication of bias is evident. The frequency of low absorbers of vitamin B_{12} among the white relatives was 33 in a total of 89, or 37 per cent. In the negro group it was 7 in a total of 17, or 41 per cent. Chi-square is very small (0.100 for one degree of freedom) and P falls between 0.70 and 0.80. It consequently seems quite admissible to pool the white and negro families in the study. Omission of the negro relatives would merely reduce the size of the group without materially altering its character, in respect to vitamin B_{12} absorption.

Analysis of the data of the relatives of patients with pernicious anemia (Figs. 3 and 4) shows that a highly significant decrease in the mean values for vitamin B_{12} absorption and an increase in the percentage of family members with abnormal or borderline tests occur with increasing age. The control group, on the other hand, shows no significant change with age, an observation which confirms previous reports (16, 31, 34).[5]

[5] It has already been pointed out that the group of relatives of probands is more heterogeneous with respect to age than the control group, and the question therefore arises,

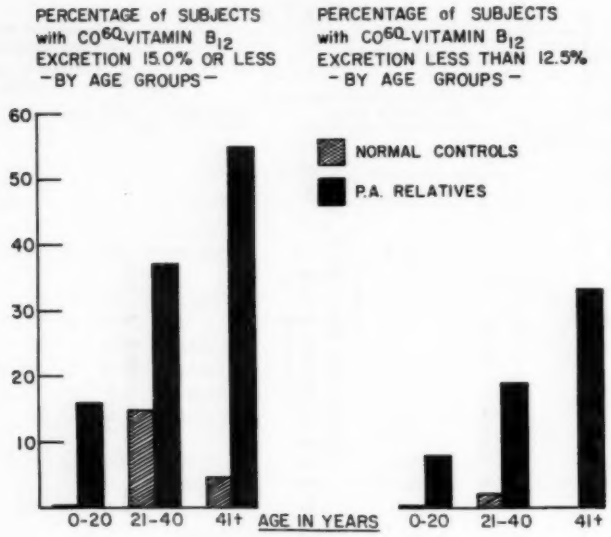

FIG. 4

In 8 relatives with confirmed low results it has been possible to repeat the test after intervals of 6 months or longer. Two of these on retesting 18 and 26 months after the initial examination show a suggestive decrease in ability to

whether the lack of an apparent regression with age in the controls is attributable to the bias with respect to age in selection of controls. If a negative regression with age really exists in the general population, the effect of the age differences between controls and relatives of pernicious anemia probands would be to obscure it. However, according to the mean ages given on p. 313, the difference should be most pronounced in the youngest age group (0–20 years) and least in the oldest age group (41+ years). Actually, as may be seen in Figure 3, the difference is greatest between the oldest age groups of controls and relatives, where the mean difference in age amounts to only one year. It may therefore be concluded that, although the results of Schilling tests on a general population randomly selected as to age are needed to provide assurance that in general, there is no relatively small negative regression in the absorption of vitamin B_{12} with age, there is no indication that any such regression exists over the interval of 34.58 years from the mean age of the youngest control group to the mean age of the oldest control group. On the other hand, the mean Schilling test value for the seven relatives 10 years of age or younger is 19.2 per cent excretion, which is almost exactly the same as that for the entire age group 0–20 years. The negative regression with age thus does not depend upon the presence or absence of young children. As for the five relatives (all of them white) actually in the same age interval as the controls within this 0–20 year-old age group, i.e., those 17–20 years old, their mean Schilling value is 16.7 per cent excretion. The number of these cases is very small, but as it stands the difference between controls and relatives is actually greater in the 17–20 year-old age group than when the younger relatives are included. It may be concluded, in answer to the question posed at the beginning of this paragraph, that the inclusion of controls less than 17 years of age would probably have made no substantial difference.

TABLE IV

Distribution of Abnormal and Borderline Schilling Test Results Among Siblings and Offspring of Patients with Pernicious Anemia

Relation to Proband	Average Age	Number Studied	Number with Schilling Test Results 15.0% or Less
Siblings	54	25	12 (48%)
Offspring	33	55	18 (32.7%)

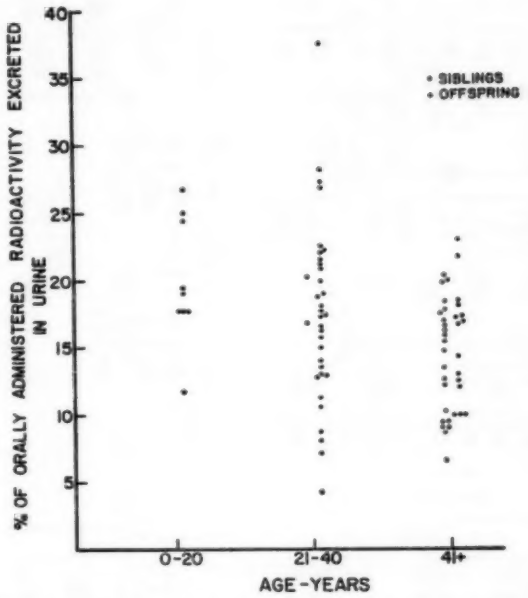

FIG. 5. Results of Schilling tests in siblings and offspring of patients with pernicious anemia in relation to age.

absorb vitamin B_{12} (Table III, Subjects 58 and 59). It is important to recognize that low absorption may occur in relatively young persons. Low values, confirmed by repeated tests, were obtained in two family members at ages 19 and 20. There is, of course, a pronounced difference in the age distribution of siblings and offspring. A higher percentage of siblings than of offspring had abnormal or borderline absorption (Table IV). The test results in both groups in relation to age are shown in Figure 5.

Distribution of test results in individual families

The results of Schilling tests in individual family groups are shown in Figure 6. To be noted is the appearance of low values in successive generations as well as in siblings. In two instances abnormal results were observed in

members of two families with a single common progenitor (Families of S. H. and M. S.). In the family of F. W. abnormal absorption was traced through three generations.

The mother of one proband (M. L.) excreted only 3.3 per cent and 5.7 per cent of the administered radioactivity in two tests. She was a woman of 86 with no evidence of pernicious anemia and with good renal function as indicated by normal urinalysis, serum urea nitrogen and urea clearance. She had

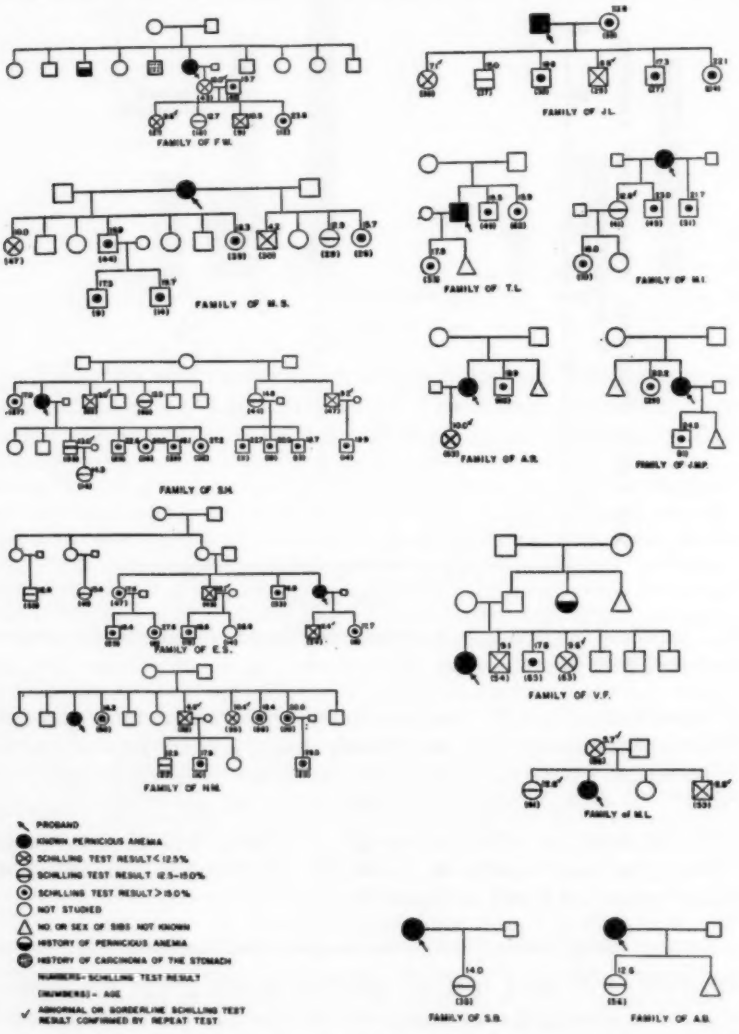

FIG. 6. Results of Schilling tests in the families of patients with pernicious anemia.

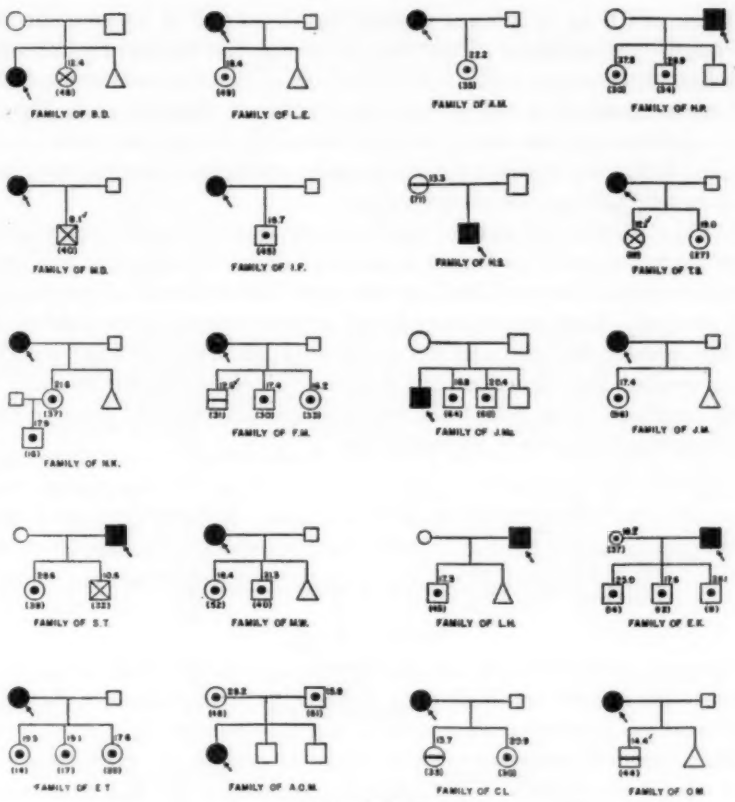

FIG. 6—Continued

had mild congestive failure associated with arteriosclerotic cardiovascular disease, but was well compensated at the time of the tests.

Proband A. O'M. is of unusual interest because she had pernicious anemia in childhood. She had developed well documented hypoparathyroidism at age five but her blood had remained normal until age 12 when typical pernicious anemia, with severe subacute combined degeneration, made its appearance. She was unable to absorb Co^{60}-vitamin B_{12} except when it was administered with intrinsic factor concentrate. Neither parent had definitely abnormal absorption, although the father's results fell into the lowest portion of the normal range.

Absorption of vitamin B_{12} by members of the families of normal control subjects with low absorption

The majority of the normal control subjects in this series and all of those studied by Callender and Evans (13) excreted more than 15.0 per cent of the

oral dose of 0.5 μg. of labelled vitamin B_{12}. It seemed of interest, therefore, to examine the members of the families of the few normal subjects whose excretion fell below this value. Relatives of two of these subjects were available and results of Schilling tests performed on them are summarized in Figure 7. It is apparent that low values were encountered in other members of these families. In the investigation of these families, additional historical information came to light and may be of significance.

D. M., a 26 year old medical student knew of no pertinent family history when he was selected as a normal control subject. He had free acid in his gastric secretion, but two Schilling tests gave low results (11.2 per cent and 12.6 per cent). Both parents were found to have normal gastric acidity. The mother excreted 30.3 per cent of the test dose, the father 15.5 per cent. The father recalled that his father had died in 1917 of "locomotor ataxia". The death certificate of the latter was examined and the recorded cause of death was "combined sclerosis of the spinal cord".

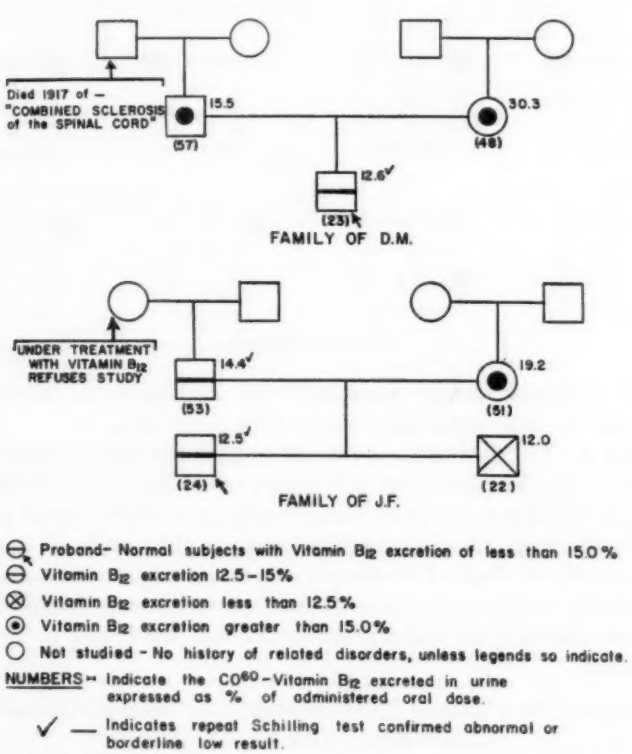

FIG. 7. Results of Schilling tests in families of two normal controls with low test values.

TABLE V

Relationship Between the Incidence of Achlorhydria and the Results of Schilling Tests in 86 Relatives of Patients with Pernicious Anemia

Result of Schilling Test	Number of Relatives Tested for Gastric Acidity	Number with Achlorhydria
>15.0%	54	4 (7.4%)
12.5–15.0%	12	3 (25%)
<12.5%	20	8 (40%)
Total group.....	86	15 (17.4%)

J. F., a 24 year old medical student thought his family history entirely irrelevant. His Schilling tests gave results of 10.5 per cent and 12.5 per cent. His mother had normal vitamin B_{12} absorption (19.2 per cent). His father had two borderline results (14.2 per cent and 14.4 per cent), and his only sibling appeared to have impaired absorption (12.5 per cent). All four of these individuals had free acid in the gastric secretion. Interview with the subject's parents revealed that the proband's paternal grandmother had, within the year, been started on treatment with vitamin B_{12} with a clinical diagnosis of pernicious anemia. She refused study to confirm this diagnosis.

Gastric acidity

Fifteen of the 86 family members tested had achlorhydria as measured by the tubeless method. In 10 instances it was possible to confirm this result by gastric intubation. Achlorhydria was more common in relatives with low values for the Schilling test, but the correlation was by no means complete (Table V). Eleven of the 15 relatives with achlorhydria had Schilling test results below 15.0 per cent. However, the majority of family members with borderline or low vitamin B_{12} absorption had free acid in the gastric secretion.

Hematologic and blood group studies

No new cases of pernicious anemia were discovered on examination of the blood of the family members. Hypochromic anemia apparently attributable to iron deficiency was encountered only once, in a woman with menorrhagia of long duration, achlorhydria and a low value for the Schilling test (Subject 43).

The frequency of blood group A in a small series of patients with pernicious anemia and their relatives is compared with a blood bank donor control group in Table VI. Only white persons were included in this study. The controls are entirely comparable in blood group distribution with samples of the Baltimore white population previously studied. The numbers in the other groups are too small to yield significant data, but the patients with pernicious anemia and

TABLE VI

Frequency of Blood Group A

	Number Examined	Number with Blood Group A
Controls (Blood Bank donors)	1834	748 (40.8%)
Patients with pernicious anemia	52	29 (55.7%)
Relatives of patients with pernicious anemia		
Total group examined	72	34 (47.3%)
Those with results of Schilling tests 15.0% or less	27	13 (48.2%)

members of their families show a trend towards a higher than normal percentage of blood group A.

DISCUSSION

In analyzing the role of genetic factors in the pathogenesis of a disorder such as pernicious anemia, in which age and environmental conditions can be shown to play a part, the problem of penetrance is unavoidable. That is to say, one must recognize that the genetic basis constitutes simply a predisposition, a susceptibility, to the development of the disease. There will inevitably be in any control group or familial group related to probands a certain number of predisposed individuals who have not, at the time of the investigation, developed clinical symptoms, and who indeed may never do so. The difficulty is lessened if, instead of dealing with the clinical disorder as phenotype, we can diagnose the condition on the basis of physiological or biochemical criteria which may show up prior to the appearance of clinical symptoms. Thus the genetic basis of one type of hemolytic anemia became apparent when it was found to be associated with sickling of the erythrocytes; further understanding emerged when it was found that carriers (heterozygotes) could be distinguished from normals, and final enlightenment arrived when it became possible to distinguish electrophoretically between hemoglobin A and hemoglobin S. The same steps might be traced for the advances in understanding of thalassemia (Cooley's, or Mediterranean, anemia). But perhaps no better parallel to the present situation under study is available than the illuminating work on hyperuricemia and its relation to the incidence of gout. The genetic factors underlying gout seemed very obscure so long as the clinical symptoms remained the sole basis of diagnosis. The study of hyperuricemia, however, made it possible to distinguish the role of sex and diet in relation to a threshold concentration of uric acid in the blood, a level above which deposition in the joints and the development of gout become highly probable. The genetic factor or factors then stood revealed as predisposing to a higher general level of uric acid in the blood of carriers of the gene than in non-carriers (38).

We must, of course, be on guard against the elementary error of assuming that because a condition is demonstrably familial it is necessarily hereditary. There are familial diseases, such as pellagra, that may be purely the outcome of shared environmental, e.g. dietary, conditions. In such cases the existence of a clearcut bimodality within the familial groups may help to establish the existence of a hereditary factor, and pedigree analysis may be conclusive.

In the present study the Schilling test provides the type of physiological diagnostic basis for the detection of the "predisposition" which has been referred to. It consequently becomes necessary to evaluate the reliability of the Schilling test. To do this one may ask the question: to what degree, in replications of the test on the same person, are variable results obtained? Although the present data are rather limited in this respect, some indication of the amount of variability is obtainable. Figure 8 presents graphically the differences between replicate tests made on the same individual. The curve is clearly a markedly skewed one, with a mode at 2.25, whereas the mean is 5.8. The significance of this may plausibly be conjectured to be as follows: The high tail of the curve, representing variations above 5.9, probably results from errors because of failure to obtain a complete urine collection on some of the tests. It is certain that such errors do occur. These limited data would then indicate that approximately 40 per cent of the variations are of this type.

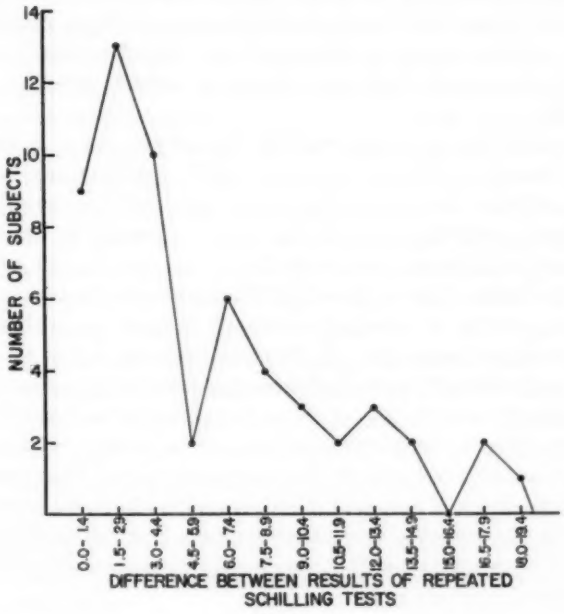

FIG. 8

The remainder of the replicate tests appear to fall into a normal frequency distribution, the mean, 2.5, corresponding closely to the mode, 2.25. One may, therefore, conclude that the inherent physiological variation in the absorption and excretion of vitamin B_{12} averages about 2.5 per cent, and that differences greater than 6 per cent result from failures in the performance of the test. It would not be valid, however, to conclude from these data that such failures occur in approximately 40 per cent of the tests. The retests in the present series were not random, but were administered in general whenever there was reason to suspect the first test of having been faulty. In other words, there were about 25 faulty tests in a total of 203 normal controls plus family members of pernicious anemia probands, or roughly 11.5 per cent faulty tests. This would mean that among persons with a Schilling test result below 15.0 per cent but who were not retested, perhaps 0.25 persons among the two normal controls and 2.4 persons among the 20 relatives would if retested prove to be above the 15 per cent level. No significant difference would result from making this amount of correction, and we may assume that the Schilling tests as recorded may be used to divide individuals arbitrarily into groups definitely low (12.5 per cent or less), borderline (12.6–15.0 per cent) and normal (above 15.0 per cent). It is encouraging evidence of the general reliability of the Schilling test and the validity of taking 15.0 per cent excretion in the urine within 24 hours as the cut-off point of the normal distribution that Callender and Evans (13) found no reading below 15.8 per cent in a group of 26 control subjects; and that among 52 achlorhydric relatives of pernicious anemia subjects, exactly half fell "within the normal range", i.e., above 15 per cent (14).

Factors other than the concentration of intrinsic factor in the gastric secretion are known to influence the result of the Schilling test. Severe impairment of absorption of vitamin B_{12} may be produced by several types of intestinal disease even though intrinsic factor is present in normal amounts. Furthermore, in the presence of renal disease vitamin B_{12} may not be excreted rapidly in the urine. The results of Schilling tests may be low in persons who are ill from a variety of causes even though there is no evidence of gastrointestinal or renal disease (Fig. 1). Whether these low values are the result of impaired production of intrinsic factor, decrease in the capacity of the intestine to absorb vitamin B_{12}, or reduced renal excretion was not determined (60). In the present study the members of the normal control and family groups were entirely well with the few exceptions noted. There is no reason to believe that the state of health of these two groups differed; hence the striking differences in the results of Schilling tests seem best explained by differences in ability to absorb vitamin B_{12}, presumably dependent upon differing rates of production of intrinsic factor.

According to Table IV, 48 per cent of sibs of pernicious anemia probands

were found with Schilling tests of 15.0 per cent or below, whereas among offspring of pernicious anemia probands the percentage with similarly lowered absorption of vitamin B_{12} was 32.7 per cent. But there is a marked age difference between sibs of probands and offspring of probands, amounting to 21 years on the average. Figures 3 and 4 show, furthermore, that there is a significant increase with age in the percentage with low absorption among relatives of the probands, although this is not evident among normal control individuals. The regression of the absorption with age is 1.5 per cent per decade (estimated from the offspring only) to 2.5 per cent per decade (estimated from sibs plus offspring). If the frequency of low absorption among offspring is accordingly corrected by adding the three cases falling between 15.0 and 16.5 per cent, the percentage with low absorption in this group becomes 38.2 per cent, which is not significantly different from the percentage found among the sibs (χ^2 = 1.68; d.f. = 1; p = .20).

This constitutes evidence that whatever genetic factor is involved is probably not, as Mosbech (65) proposed, a simple recessive one, for a recessive trait would be expected to show up in somewhat less than 25 per cent of the sibs of a proband; but unless the trait was very common in the population most mates of probands would not be heterozygous, and the frequency among offspring would consequently be lower than among sibs. From the controls (Table III) it may be seen that among 97 persons there were three who each had one Schilling test below 12.5 per cent and four more persons with Schilling tests definitely below 15.0 per cent and probably to be included in the low absorption group. Three other borderline cases, with one test above and one below 15.0 in each instance would if added to the above group represent the maximum number of low absorbers in the control group. One thus arrives at a maximum frequency of low absorbers in the general population amounting to about 10 per cent. If the trait were a simple recessive, the allele frequency would then fall at or below .32 (more probably around .27); and the frequency of heterozygotes in a population in genetic equilibrium would fall at or below 43.5 per cent, and probably close to 40 per cent. Hence, even if the trait were attributable to a very common recessive gene, the probability of obtaining homozygous recessive offspring from a mating between a homozygote and a non-affected person would be considerably below .50 (more precisely, 20 to 22 per cent or below). Since the frequency of low absorbers in the general population is probably really nearer 5 per cent than 10 per cent, we may conclude that the evidence is rather against the participation of a simple recessive gene. A larger, and as to age, more representative group of controls will be required for final evaluation of this hypothesis, and for a more reliable determination of the frequency of genetically determined low vitamin B_{12} absorption in the general population.

One might next assume that low absorption is caused by a gene in hetero-

zygous condition, the homozygous state being pernicious anemia. This would be analogous to the situation in thalassemia and in sickle cell anemia. But this hypothesis can be ruled out, for if it were true, then every offspring of a pernicious anemia proband should be heterozygous and characterized by low absorption of vitamin B_{12}. Table II and numerous pedigrees show that pernicious anemia probands commonly have many offspring with definitely normal vitamin B_{12} absorption. In fact, in 26 families in which the mate of the pernicious anemia proband was not tested there were only six families with no normal offspring; and in two families in which the mate of the proband was tested and was definitely normal in vitamin B_{12} absorption, both families included normal offspring. The total progeny from these 28 families included 10 offspring who were low absorbers, nine who were borderline, and 36 definitely normal (i.e. above 15.0 per cent excretion). Moreover, the frequency of offspring with low absorption was actually somewhat higher in families without a parent with pernicious anemia, but having instead one parent with a low or borderline absorption of the vitamin. One may conclude, therefore, that clinical pernicious anemia does not increase the probability of having offspring with low vitamin B_{12} absorption over the probability already present in matings where low absorption is present in one of the parents.

A third hypothesis would be that low vitamin B_{12} absorption depends on the presence of a heterozygous gene which is a necessary but not sufficient precondition for pernicious anemia. The data fit this hypothesis very well. The 38 families with one parent either a proband with pernicious anemia or a low or probably low absorber of vitamin B_{12} yield a total of 45 normal offspring, 13 with borderline absorption (12.6–15.0 per cent), and 16 with definitely low absorption. The families with a parent in the borderline region produced four normal, one borderline, and two low absorbers, a proportion no different from families with a parent definitely a low absorber or families with a pernicious anemia parent. If, then, the plausible assumption is made that the borderline families are in most instances properly to be reckoned with the low absorbers, there is a total ratio of normal offspring to low absorbers of 45:29. On the basis of this hypothesis, equal frequencies would be expected, subject to the correction for age. The ratio of observed to expected (29:37) might therefore indicate a penetrance of 78 per cent. But the cases involved are so few that the difference from a 1:1 ratio, to be expected on the basis of this hypothesis, is actually not statistically significant ($\chi^2 = 3.47$; d.f., 1; $.10 > p > .05$), even without the correction for age.

Another test of this hypothesis, and one particularly useful in distinguishing a simple dominant from a polygenic condition, is to determine the proportion of affected persons who have one, both, or neither of their parents similarly affected. In the case of a single dominant gene, especially if it is relatively rare—which is generally the case—an affected person should have a single

parent affected. If the condition were polygenic, in the simplest case of two or more factors without dominance, acting additively and without epistasis, cases where an affected person has two affected parents and cases with no affected parents should be found with some frequency. Unfortunately, in obtaining the present family data it was rarely possible to test both parents of pernicious anemia subjects or of their relatives with low vitamin B_{12} absorption. Nevertheless, a check of the 29 persons in these families for whom at least one parent was tested shows 28 with a parent also low in vitamin B_{12} absorption; and only one exceptional case had a parent with an excretion of 15.8 per cent, or barely above the chosen borderline. Among the cases in which *both* parents were tested, 6 persons possessed one affected parent and one unaffected parent; one person, already referred to, had two "normal" parents but one of them was close to the borderline (15.8 per cent). In the two families of control individuals with low absorption, both parents were tested. Two individuals had a single affected parent; one had two "normal" parents, but again one of these was actually borderline (15.5 per cent). Altogether, in a total of 32 persons with a tested parent, 30 had an affected parent, 2 were dubious exceptions. In a total of 10 persons (included in the foregoing group) with 2 tested parents, there were no cases where both parents were low absorbers. The evidence, although inadequate to settle the point, is thus strongly suggestive of a single dominant factor rather than a polygenic situation. If modifiers other than environmental factors are present, they probably exert a relatively minor influence, perhaps upon the decline in absorption capacity with age (see below).

According to this hypothesis, we may assume that a single dominant autosomal gene is responsible for the abnormality of the gastric mucosa which brings about low absorption of vitamin B_{12} of the type that occurs in pernicious anemia. This gastric change results in a deficiency of intrinsic factor. However, in most persons who have a genetically conditioned abnormally low absorption of vitamin B_{12}, the gastric lesion does not become sufficiently severe that pernicious anemia develops. Our data suggest that there is no regular relationship between the times at which hydrochloric acid and intrinsic factor can no longer be produced by the affected stomach, a concept which is supported by other evidence (49). Even when production of intrinsic factor has fallen to a low level, the onset of pernicious anemia may be long delayed, depending upon the size of the vitamin B_{12} stores in the tissues, the diet, and other factors influencing the requirement for the vitamin. The mother of one proband (M. L.) in the present study was found at the age of 86 to have a Schilling test as abnormal as those encountered in patients with pernicious anemia, yet she remained well.

The hypothesis we thus favor on the basis of these data postulates a hereditary basis for pernicious anemia which should be distinguished from earlier

suggestions that pernicious anemia is the consequence of a dominant gene, perhaps with reduced penetrance (61, 69, 70, 78). In pernicious anemia achlorhydria is usually present. The dominant factor postulated on the basis of the present study produces a significantly lowered absorption of vitamin B_{12} quite frequently in the absence of achlorhydria (Table VI). Longitudinal studies of intrinsic factor secretion in sibs or offspring of pernicious anemia subjects, correlated with studies of acid production and with gastric biopsies are needed to delineate the factor responsible for the increasing incidence of pernicious anemia with advancing age. Further information might thereby be obtained concerning the role of genetic and other influences including the aging process itself. Recent studies with biopsies of the gastric mucosa in patients with *early* pernicious anemia have shown that the anatomical lesion may be slight and that atrophic changes may not be apparent (57). It is a considerable defect in the otherwise excellent study of Callender and Denborough (14) that they limited studies of vitamin B_{12} absorption by means of the Schilling test to relatives *with* achlorhydria, and consequently missed the important fact that low absorption of vitamin B_{12} occurs in the families of pernicious anemia subjects quite frequently in the absence of achlorhydria. Their data are consequently of little value in the attempt to distinguish the several genetic and nongenetic factors involved in the incidence of pernicious anemia. The hypothesis of what Callender and Denborough called a "pre-pernicious anemia", "larval pernicious anemia", or "latent pernicious anemia" is nevertheless very similar to the present one, except for the implication conveyed by the first two cognomens that there is some steady or inevitable progression from the status of a low absorber of vitamin B_{12} to pernicious anemia. According to our hypothesis, the latent state is a necessary but not sufficient condition. It may remain benign throughout life. Accessory factors are necessary to convert the latent or predisposed condition to the diseased one.

There are complications, and probably our own hypothesis is oversimplified. One complication relates to the regression in absorption capacity with age. The results of the Schilling tests in the normal control group offer a good approximation of a normal frequency distribution (Fig. 2). The distribution among the relatives of pernicious anemia probands is clearly bimodal. But even the "normal" portion of the latter distribution averages well below the average of the normal controls (Figs. 2 and 3). From the age distribution of these two groups (Figs. 3 and 4) it appears clear that only the families of the pernicious anemia patients show a significant regression with age in the magnitude of the Schilling test value. This is unlike the situation in respect to achlorhydria, wherein normal controls as well as relatives of pernicious anemia subjects show a regression with age (14, 17). The age-related loss of ability to absorb vitamin B_{12}, shown by many relatives of pernicious anemia patients in this study, as well as the well-documented increase in the incidence of pernicious

anemia with age and the many instances in which evidence of gastric abnormality (e.g., achlorhydria) has preceded by years the onset of pernicious anemia, may thus be distinct from the genetic factor responsible for the bimodality of the distribution of Schilling tests among the relatives; that is to say, in some persons the genetic predisposition to low absorption may be manifested irrespective of age, in others onset may be age-influenced. The undetermined familial factors responsible for this age regression in absorption capacity for vitamin B_{12} may possibly be the same as those provoking achlorhydria, with its well-established age-regression; or they may be different. They may be genetic, non-genetic, or both.

Pernicious anemia is extremely rare in childhood. If adult pernicious anemia develops in persons who are heterozygous for an abnormal gene, childhood pernicious anemia might represent the result of homozygous inheritance. This hypothesis is not supported by study of the family of the one child in our group of probands. This child also had hypoparathyroidism, a disease which has been associated with childhood pernicious anemia in two other cases (47, 76). The relationship between the two diseases as well as the cause of the early onset of pernicious anemia remains obscure.

In the absence of more extensive genetic information, it would be premature to speculate further. Much work will need to be done to clarify the picture. In particular, pedigrees are needed in which two low absorbers have mated and produced progeny. Callender and Denborough (14) reported two matings involving pernicious anemia in both parents, but in neither family were Schilling tests administered to the progeny. In one family both sons were found to have normal acid secretion and normal serum vitamin B_{12} levels, along with other normal tests, and there is accordingly a strong presumption that they had normal vitamin B_{12} absorption. In the other family, the only tested offspring, a son, had achlorhydria but refused to take other tests relating to B_{12} absorption. These cases support the genetic interpretation given in the present paper, since they indicate that a mating of two pernicious anemia subjects does not invariably produce progeny with low B_{12} absorption. Additional information ought also to be collected regarding the incidence of low vitamin B_{12} absorption in a number of different populations. Longitudinal studies to determine the frequency with which low absorbers develop pernicious anemia would be extremely valuable.

Meanwhile it is definitely not worthwhile to speculate about the possible reasons for the association of pernicious anemia or low vitamin B_{12} absorption with anatomical features, fair complexion, blue eye color, blood group A, or secretor status—all of which have been suggested—until something more is known regarding the frequency of the low absorption factor in different populations and races. It should be pointed out, however, that pernicious anemia has its highest incidence among North European populations in which these

physical characteristics are most common; and the data in Table V, which suggest an elevated frequency of blood group A in the *normal* relatives of pernicious anemia patients, as well as in those with low absorption of vitamin B_{12}, imply that the association is an ethnic one rather than one of close genetic linkage or pleiotropy.

From the genetic point of view it is well, in concluding, to re-emphasize the point made by Neel (70, 71, 72) regarding the great value to clinical medicine of detecting the carriers of inherited diseases. Not only will the detection of those predisposed to pernicious anemia enable the clinician to be forewarned and forearmed, but also further study of these individuals as they age will undoubtedly make possible a further clarification of the interacting causes of this disease. In particular, the identification of intrinsic factor, now that its secretion is shown to be genetically determined, may become easier, until eventually the exact nature of the genetic block in carriers of the gene, the basic nature of the "inborn error of metabolism", is elucidated, as in other examples (galactosemia, phenylketonuria, favism) in recent years.

SUMMARY

One hundred and six relatives of 34 patients with pernicious anemia were tested for their ability to absorb tracer doses of Co^{60}-vitamin B_{12} by the method of Schilling. Ninety-seven healthy control subjects were similarly studied. A significantly greater proportion of the family members than of the controls had low test results. There was also a highly significant increase in the number of family members with abnormally low vitamin B_{12} absorption with increasing age. The control group showed no significant variation in the results of the Schilling test with age. The distribution of test results in the individual family groups is presented. The data indicate that predisposition to pernicious anemia is inherited, most probably as the effect of a single dominant autosomal factor. The hypothesis is excluded that pernicious anemia is the homozygous effect of a gene that in heterozygous state produces low absorption of vitamin B_{12}. The alternative hypotheses that low absorption of vitamin B_{12} is attributable either to a single recessive gene or to multiple genetic factors are less in accord with the data than the hypothesis of a single dominant factor.

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ABSTRACTS OF PAPERS PRESENTED BEFORE THE MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

March 9, 1959

ENZYMATIC STUDIES OF THE CLOT LYSING SYSTEM OF THE BLOOD

PHILIP S. NORMAN

Spontaneous clot lysis is an abnormal phenomenon which may be observed in the blood of humans with febrile infections, chronic liver disease, cancer, traumatic injuries, shock or burns. It may be induced experimentally by administration of bacterial endotoxins, ACTH, or epinephrine and is seen during anaphylaxis. Clot lysis is induced by a proteolytic enzyme called plasmin which digests the fibrin matrix of clots. Plasmin is present in normal blood as an inactive precursor called plasminogen, a portion of which is converted to the active enzyme in the foregoing conditions. A number of sources of activators of plasminogen are known and include urine, tissues, bile, staphylococci and streptococci. In addition, plasmin itself is an activator. Where studied, these activators act by a partial proteolytic degradation of plasminogen uncovering the active enzymatic site. The streptococcal activator, streptokinase, is an exception and seems to require a prior reaction with some component of the serum protein before conversion to plasmin takes place. A number of purification techniques have been tried and this component has been entirely inseparable from plasminogen. It is suggested that the plasminogen molecule is degraded in two steps, and that the first product catalyzes production of the second and final product, i.e., plasmin.

Normal blood also contains two inhibitors of plasmin. One is an $\alpha 2$-globulin and reacts immediately with plasmin, but reversibly; the second is an $\alpha 1$-globulin and combines slowly but firmly with plasmin at a rate that depends on temperature. The properties of these inhibitors are such as to allow digestion of a clot by plasmin to go to completion before the enzyme is inhibited. The serum proteins concerned with the activation and subsequent degradation of plasmin might collectively be called the plasmin system. What physiological function the plasmin system subserves is undetermined but its properties make it ideal for removal of intravascular clots.

THE CENTRAL SITE OF PYROGENIC ACTION OF BACTERIAL ENDOTOXINS

JAMES W. WOODS,[1] PHILIP BARD AND DAVID L. GLANCY

The literature reveals a number of attempts to determine the cerebral site of action of bacterial endotoxins (1, 2). In those studies efforts were made to induce fever in laboratory animals following the placement of restricted or massive lesions in the hypothalamus or following transection of the brainstem at various levels. In the latter case, only acute preparations were studied. Although various and bizarre disturbances of temperature regulation were produced in such ways, the results to date have been inconclusive. Actually, convincing evidence that such toxins do indeed act directly upon the brain is of very recent origin (3, 4).

During the past 18 months we have had in our laboratory several hemidecorticate, hy-

[1] Senior Fellow of the USPHS.

pothalamic, and decerebrate (mesencephalic or pontine) cats. Although these animals were prepared primarily for study of endocrine phenomena, they afforded an excellent opportunity to make observations on temperature regulation. Particular attention has been focused upon their ability to develop spontaneous and experimentally induced fevers.

The preparation of hypothalamic or decerebrate cats was essentially the same in the first stage of operation: all cerebral cortex, basal ganglia, and dorsal thalamus were removed by aspiration. This completed the ablation in the case of the hypothalamic animal. In decerebration, this ablation was followed by aspiration of a wedge of brainstem tissue which isolated the remaining forebrain (consisting of hypothalamus only) from the brainstem. The level of section of the brainstem varied from the anterior mesencephalon to the anterior one-third of the pons.

Such preparations as these require diligent nursing care but, given adequate care, they can be kept alive and healthy indefinitely. The average survival time of the decerebrate cats was over four months. No experiments are considered which were carried out prior to the 60th postoperative day. Only one hypothalamic cat is reported here. He is now 112 days postoperative.

We have to date carried out 18 experiments on 10 normal cats, 17 experiments on 7 decerebrate cats, and 3 experiments on one hypothalamic cat. The general plan of these experiments was to record deep colonic temperature for two or more hours prior to the injection of the pyrogen. During this control period at least two samples of venous blood were taken and leucocytes were counted immediately. At the end of the control period 0.01–0.05 ml. of typhoid vaccine[2] was injected intravenously. Colonic temperature was taken and leucocyte counts made at appropriate intervals during the succeeding 10 to 17 hours.

In all normal cats and in those with unilateral forebrain ablations, injection of the toxin was followed by a characteristic series of events: (1) leucopenia, (2) a rise in colonic temperature, (3) leucocytosis, (4) defervesence.

In the decerebrate cats vaccine injection was invariably followed by leucopenia, but no evidence of a rise in colonic temperature has ever been observed. The terminal leucocytosis occured only under certain conditions.

Since decerebrate cats are poikilothermic, the design of an experiment to detect a pyrogenic response is complicated. Four different experimental designs were used: a) injecting the vaccine sometime after disconnecting the cage heaters and after a rate of fall of body temperature had been established, b) injecting after reducing the cage heat to an amount which would allow a very slow rate of fall of body temperature, c) injecting after the cage heaters had been disconnected long enough (24 or more hours) to allow colonic temperature to plateau at a low level (around 30°C.), d) injecting with cage heat on normal automatic control (which causes body temperature to oscillate 0.5° above and below 38.3° C.). No evidence of a pyrogenic reaction was ever observed, regardless of the conditions of the experiment. It is interesting to note that terminal leucocytosis occurred only under those conditions in which colonic temperature was maintained at normal level.

In the one hypothalamic cat studied so far, the magnitude and the time course of the changes in colonic temperature and leucocyte count were indistinguishable from those observed in normal controls. This cat has also had at least one spontaneous fever which was accompanied by symptoms of a respiratory tract infection. The fever and the symptoms subsided within 48 hours of initiation of penicillin therapy.

These conclusions appear to be inescapable: (1) that cats in which the hypothalamus has been disconnected from the brainstem are incapable of responding to typhoid vaccine

[2] The typhoid vaccine was generously supplied by Drs. Philip Norman and Ivan L. Bennett. The same batch of material was used in all experiments.

with a rise in body temperature although they do undergo characteristic changes as regards white cell count in peripheral venous blood, (2) that the level of truncation of the brain-stem is of no consequence in so far as the responses under consideration are concerned, and (3) that, since cats without cerebral cortex, basal ganglia, or dorsal thalamus respond with a normal fever to such toxins, some hypothalamic area is essential to the production of fever by typhoid vaccine.

The assistance of Mr. T. A. Feild, III in the determination of leucocytes is gratefully acknowledged.

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THE ROLE OF COMPLEMENT IN THE MEDIATION OF ALLERGIC TISSUE RESPONSES

Abraham G. Osler

Two experimental models of anaphylactic reactions have been investigated with respect to the participation of complement in the induction of the tissue injury associated with these allergic manifestations. In studies of passive cutaneous anaphylaxis (PCA), the following correlative findings have been observed.

a) The intensity of the allergic reaction of the immediate type varies directly with the quantity of available antigen, antibody, or a serum factor closely resembling hemolytic complement.

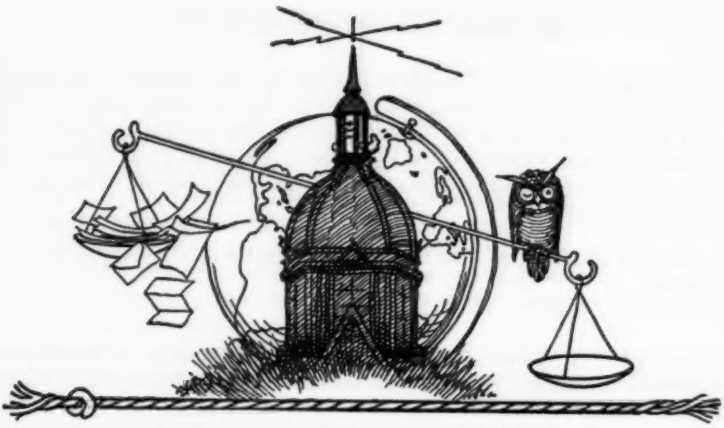
b) These reactions may be enhanced by the injection of hemolytically active serum into normal rats.

c) Rats which have been rendered deficient in circulating complement are relatively refractory to the induction of PCA reactions.

d) The cutaneous reactivity of these complement deficient animals may be partially restored with serum reagents possessing hemolytic activity, or third complement component activity.

3) There is a marked parallelism in the *in vitro* efficacy of an immune system to fix complement and to invoke PCA reactions.

Findings of a generally similar nature have been obtained in studies of anaphylatoxin, a product of complement fixation. In the test tube or in the animal body, the interaction of antigen with antibody in the presence of fresh serum leads to the simultaneous loss of hemolytic potency and genesis of anaphylatoxin, provided all of the known complement components have been involved in the reaction. Anaphylatoxin has been assayed in terms of capillary permeability enhancement and smooth muscle contraction, the two cardinal tissue lesions of allergic reactions of the immediate type. The importance of these phenomena in the immunopathology of various disease processes remains to be evaluated.



BOOK REVIEWS

The Anatomy of the Nervous System. Its Development and Function, 10th edition. By STEPHEN WALTER RANSON AND SAM LILLARD CLARK. 622 pp., $9.50. W. B. Saunders Co., Philadelphia, Pa.

Ranson's textbook, now in its tenth edition, needs no introduction. Since 1920 it has been a standard work for undergraduates, and a ready source of anatomical information for the neurologist. In the present revision, Dr. Clark has added considerable new material on cerebral and cerebellar representation and has introduced some consideration of electron microscopy. There is a stronger functional orientation than in earlier editions—some justified physiologically, some less so. Many areas are considered in less detail than some advanced students would wish—indeed, blood supply remains woefully weak. These shortcomings, however, are mainly those of any precise manual of this size. It serves a very useful purpose.

J. W. MAGLADERY

Color Atlas of Morphologic Hematology with a Guide to Clinical Interpretation, Revised edition. By GENEVA A. DALAND. Edited by THOMAS HALE HAM. 72 pp., plus 30 pp. of illustrations and legends, $6.75. Harvard University Press, Cambridge, Mass.

This is a beautifully illustrated companion book to "A Syllabus of Laboratory Examinations in Clinical Diagnosis" by one of the most experienced cytologists in hematology. Geneva Daland has had an indispensable role for years in many of the hematological studies carried on at the Throndike Memorial Laboratory. She has brought to fruition in this book a wealth of experience based on very careful observations. The book has been edited by Dr. Thomas Hale Ham in his typically meticulous manner.

This is a very valuable contribution.

PHILIP F. WAGLEY

Physical Diagnosis. The History and Examination of the Patient. By JOHN A. PRIOR AND JACK S. SILBERSTEIN. 388 pp., $7.50. The C. V. Mosby Co., St. Louis, Mo.

The reviewer wholeheartedly recommends this textbook of physical diagnosis for use by medical students. The emphasis on history-taking, in addition to the techniques of examination which are a standard part of physical diagnosis, is laudable. The neurologic,

pediatric, skeletal, otolaryngological and other special aspects of the examination are discussed by appropriate colleague-specialists of the authors at Ohio State University College of Medicine. The book is well illustrated. Entirely conventional in its approach, the book is as good as, or better than, any other now available on the subject.

VICTOR A. MCKUSICK

Clinical Orthopaedics No. 12. Rehabilitation. Edited by ANTHONY F. DEPALMA. 327 pp., $7.50 single copies. J. B. Lippincott Co., Philadelphia, Pa.

Volume 12 of *Clinical Orthopaedics*, like the preceding issues of this series, consists of articles by various authors on practical clinical orthopaedic subjects. The first half of this volume is concerned with rehabilitation and the remainder is given to general orthopaedic problems such as fat embolism, fractured hips and miscellaneous case reports. The articles on rehabilitation are written by prominent men in this important and newly emphasized phase of medicine, such as McBride, Kessler, Aitken, and Hoover. These articles review their experiences, prejudices and practical solutions to practical problems in the art of medicine. Thus, many of the articles are essays or commentaries and as such will be of considerable interest to orthopaedists facing these same problems. The review character of many of the articles may open new vistas to house-officers whose parent institutions may not have given them adequate background in certain fields to relish the pursuit of these unfamiliar fields in specialty journals. However, the house-officer will find the lack of factual data and reasonable proof of conclusions a poor place to build the basics of his education. We imagine the medical student will be too remote from the practical problems to appreciate this book.

The majority of the factual material has been presented before and elsewhere, but there are several interesting original articles such as that by Urist on "The Repair of Articular Surfaces Following Arthroplasty of the Hip".

In general, the quality of the articles as well as their readability, varies widely and selection of the wheat from the chaff will have to be done by the reader instead of the editor.

The format, illustrations, and binding are good and each article has a summary in interlingua.

GEORGE W. SETTLE

Surgery in World War II. Vol. I, Neurosurgery. Prepared and published under the direction of MAJ. GEN. S. B. HAYS, The Surgeon General, U. S. Army. Editor in Chief COL. JOHN BOYD COATES, JR. Editors for Neurosurgery R. GLEN SPURLING AND BARNES WOODHALL. Assoc. Editor ELIZABETH M. MCFETRIDGE. 466 pp., $5.00. Government Printing Office, Washington, D. C.

This volume is one in a large series devoted to surgery in World War II and will be followed by a second volume of neurosurgery in the near future. Volume I includes a brief historical sketch of World War I neurosurgery and several chapters devoted to a detailed account of the administrative system evolved to care for neurosurgical casualties from the battlefield to the large and frequently specialized general hospitals in this country.

The American Board of Neurological Surgery began functioning in the year the war began. Therefore, much of the work load in combat zones fell to general surgeons who had had some training in neurosurgery and later to younger general surgeons who received an intensive 30 days course in the specialty and three or four months of preceptorship before being sent overseas. The magnitude of the personnel problem is emphasized by the fact that there were 200 board certified neurosurgeons in 1942 and 22,000 neurosurgical beds in the Zone of the Interior alone in 1945.

Although the number of neurosurgical casualties was grossly underestimated the per cent of neurosurgical cases among battlefield casualties was accurately predicted and averaged about 10 per cent. These were subdivided into 5 per cent peripheral nerve injuries, 4 per cent cranio-cerebral trauma, and 1 per cent spine injuries. However, this is an erroneous index of the incidence of brain wounds in combat because of 1,000 autopsies performed on men who were mortally wounded on the battlefield 40 per cent either definitely or probably died of intracranial injuries.

The remainder of the book is devoted to the management of head injuries. Whereas closed head injuries constitute the vast majority of problems in cranio-cerebral trauma in civilian practice, the converse is true in wartime neurosurgery. One of the principal contributions of this volume is a presentation of the method of treatment of these wounds, well illustrated with drawings and photographs. Experiences in World War II in techniques of cranioplasty and the meticulous evaluation of posttraumatic epilepsy are presented in detail.

Many aspects of pathology and physiology are discussed, but the principal purpose of this volume is to present the clinical neurosurgical experience of World War II and as such it may serve as a handbook should the need arise again for wartime neurosurgeons. It is unfortunate that 13 years have been required for publication.

THOMAS W. LANGFITT

Physiology of Spinal Anesthesia. By NICHOLAS M. GREENE. 195 pp., $6.00. Williams & Wilkins Co., Baltimore, Md.

For the first time a monograph concerning spinal anesthesia has been published which concerns itself not with the clinical aspects, but almost purely with the basic pharmacology and physiology underlying the use of spinal anesthesia.

Dr. Greene has made an extensive review of the literature, and has put together a book which is some of the most worthwhile reading in anesthesiology that has come to my attention in three or four years. Dr. Greene has seen fit to approach the subject of physiology of spinal anesthesia by studying its effects on the various systems of the body, namely, the central nervous system, cardiovascular system, renal, hepatic, endocrine systems, and the like.

There is an especially interesting chapter on obstetrical physiology in relation to spinal anesthesia. This is an area which is filled with more lore than learning, and deserves a rational approach. The reading of this book gives one an excellent understanding of what he may be doing by giving spinal anesthesia, in a way that no description in a clinically oriented monograph would do. It is a worthwhile addition to the bookshelf of any anesthesiologist or surgeon.

DONALD W. BENSON

Practical Dermatology, 2nd edition. By GEORGE M. LEWIS. 363 pp., $8.00. W. B. Saunders Co., Philadelphia, Pa.

This is the second and revised edition of a book Dr. Lewis first published in 1952. It is exactly the same size, that is, ten by six and one half inches, but contains more pages three hundred and sixty three pages as against three hundred and twenty eight in the first edition. Therefore it is still a small book as a representative of dermatological texts.

Written primarily for medical students, the book is certainly the best of its class I've ever seen. There are twenty six chapters organizing the subject matter concisely and understandably. The commoner skin diseases are described fully and well with good photographs, the rarer diseases in shorter form.

I can find fault only with the emphasis which Dr. Lewis places upon dietary measures in the treatment of acne vulgaris, a disease which in my opinion is entirely of endocrine origin.

A bibliography is appended.

H. HANFORD HOPKINS

Cold Injury, Transactions of the Fifth Conference, March, 1957. Edited by M. IRENE FERRER. 341 pp., $5.95. Josiah Macy Foundation, New York, N. Y.

This book is, in all likelihood, of greater interest to the scientific reader because of its approach than its content. The general content is extremely well presented, but at the same time very specialized. The approach, however, is one which makes it possible for the reader to actually inject himself into the conference itself.

Dr. Ferrer has done an excellent job of presenting the conference in a fashion which leaves out none of the intentness or excitement of the moment. There are only seven main speakers, and they discuss a variety of topics, such as: animal adaptation to cold, human acclimatization to cold, some observations on ventricular fibrillation in acute hypothermia and energy metabolism in cold. The meetings are obviously held in a very informal fashion, for the speaker is interrupted frequently with searching questions, and more often than not, a very revealing discussion results.

The marvelous description of the human acclimatization to cold by Dr. Kaare Rodahl makes excellent reading for someone not even interested in cold injury. Dr. Benjamin Covino, long interested in hypothermia and its clinical application, discusses some very interesting aspects of ventricular fibrillation in acute hypothermia. This should be of interest to the clinical surgeon and to those interested in utilizing hypothermia as a research tool.

In all, the book is an extremely stimulating one for anyone interested in the effects of lowered temperatures on biological activity.

DONALD W. BENSON

Physiological Bases of Psychiatry. (American Lecture Series). Compiled and Edited by W. HORSLEY GANTT. 344 pp., $10.50. Charles C Thomas, Springfield, Ill.

The bulk of this monograph is made up of the eighteen papers presented at the twenty-fifth anniversary of the establishment of the Pavlovian Laboratory of The Johns Hopkins University. Five papers from Howard Liddel's Behavior Laboratory at Cornell University complete the volume.

Both laboratories are the pioneers in this country in the study of behavior by Pavlovian methods. The diversity of the papers collected here illustrates the wide applicability of this approach to psychophysiological research. The conditional reflex methodology is, of course, particularly attractive to those who wish their study of behavior to yield verifiable quantitative data.

Gelhorn in his paper "Objective Psychiatry", sounds the keynote of much of the book in his plea for adherence to sound principles of scientific investigation by those engaged in psychiatric research. Although not all behavioral research is as subject to careful testing of its conclusions as most of the material presented here, psychiatric research in general could well follow the example of these investigators in their concern with and appreciation of the limits of error in the methods used.

The clinical psychiatrist will find the papers of variable interest and may have difficulty following some of them because of the terminology and abbreviations. However, many of them should be extremely interesting not only to psychiatrists and psychologists, but to

any one interested in some ingenious research. Richter's "The Phenomenon of Unexplained Sudden Death in Animals and Man", and Moore's "Conditioning and Stress in the Newborn Lamb and Kid" are models of clear exposition. Dykman and Gantt's "Cardiovascular Conditioning in Dogs and Humans" is a particularly beautifully planned and executed piece of research in which the sensitivity of the conditional heart rate and blood pressure response is studied and related to the differentiated motor conditional response.

The long list of publications from the Pavlovian Laboratory at the end of the volume is a tribute to the widespread influence of Dr. Gantt on his many collaborators.

JOSEPH H. STEPHENS

Vascular Surgery. By GEZA DE TAKATS. 726 pp., $17.50. W. B. Saunders Co., Philadelphia, Pa.

In Saunders' publication "Vascular Surgery", Dr. de Takats records in detail his vast experience in the field of the peripheral vascular disease. The chapters are well developed, yet in some, subject matter is stressed according to the author's interest rather than according to its clinical importance. The discussions of arterial injuries and of degenerative arterial lesions are excellent. The treatment of essential hypertension is complete but more fitting for a medical text. A chapter on portal hypertension is not included.

The section on surgical procedures is well planned, but the diagrammatic sketches are too brief and the prose too meager to be beneficial unless one has had much surgical training. This book will be appreciated best by an experienced surgeon. For the student and the general practitioner use of the complete bibliography should increase its value.

JOHN N. CLASSEN

Cyclopropane Anesthesia, 2nd edition. By BENJAMIN H. ROBBINS. 293 pp., $9.00. Williams & Wilkins Co., Baltimore, Md.

In 1939, Dr. Benjamin Robbins published his first edition of "Cyclopropane Anesthesia." At that time it was the only monograph on cyclopropane. Through the years it has remained as such. As Dr. Robbins points out in his preface to this book, the use of cyclopropane has increased several fold, and actually from 1951 to 1956, increased by a factor of 2. The importance of cyclopropane as a tool for the clinical anesthesiologist cannot be overestimated. Most reserve it for the most difficult situations and feel strongly about its indications in specific disease entities.

This second edition of Dr. Robbins' monograph brings up-to-date the use of cyclopropane in conjunction with such things as relaxants, levels of anesthesia as they can be determined by electroencephalography in respect to cyclopropane, and up-to-date data on the circulatory effects of the agent, and a review of clinical indications and contraindications of the drug that have been published since the first edition.

The book is a gold mine of information for the anesthesiologist concerning cyclopropane anesthesia, being complete in its coverage of the pertinent literature, and presented in an organized fashion.

DONALD W. BENSON

BOOKS RECEIVED FOR REVIEW

Disease of Laboratory Primates. By THEODORE C. RUCH. 600 pp., $7.50. W. B. Saunders Co., Philadelphia, Pa.

Diseases of Metabolism, 4th edition. Edited by GARFIELD G. DUNCAN. 1104 pp., $18.50. W. B. Saunders Co., Philadelphia, Pa.

Group Processes. Transactions of the Fourth Conference Oct. 1957. Edited by BERTRAM SCHAFFNER. 266 pp., $4.50. The Josiah Macy, Jr. Foundation Publications, New York, N. Y.

Maternity. A Guide to Prospective Motherhood. By FREDERICK W. GOODRICH, JR. 130 pp., $1.75. Prentice-Hall, New York, N. Y.

Pediatric Neurology. By STANLEY S. LAMM. 495 pp., $12.90. Landsberger Medical Books, New York, N. Y.

The Plasma Proteins. Clinical Significance. By PAUL G. WEIL. 133 pp., $3.50. J. B. Lippincott Co., Philadelphia, Pa.

The Psychiatric Aide. A Textbook of Patient Care, 2nd edition. By ALICE M. ROBINSON. 200 pp., $3.50. J. B. Lippincott Co., Philadelphia, Pa.

A Short Practice of Surgery, 11th edition. By HAMILTON BAILEY AND MCNEILL LOVE. 1389 pp., $18.00. J. B. Lippincott Co., Philadelphia, Pa.

Surgery of the Prostate. By HENRY M. WEYRAUCH. 535 pp., $15.00. W. B. Saunders Co., Philadelphia, Pa.

Surgical Pathology, 2nd edition. By LAUREN V. ACKERMAN in collaboration with HARVEY R. BUTCHER JR. 1096 pp., $15.00. The C. V. Mosby Co., St. Louis, Mo.

Textbook of Microbiology, 17th edition. By WILLIAM BURROWS. 954 pp., $14.00. W. B. Saunders Co., Philadelphia, Pa.

Textbook of Surgery, 3rd edition. Edited by H. FRED MOSELEY. 1336 pp., $17.00. The C. V. Mosby Co., St. Louis, Mo.

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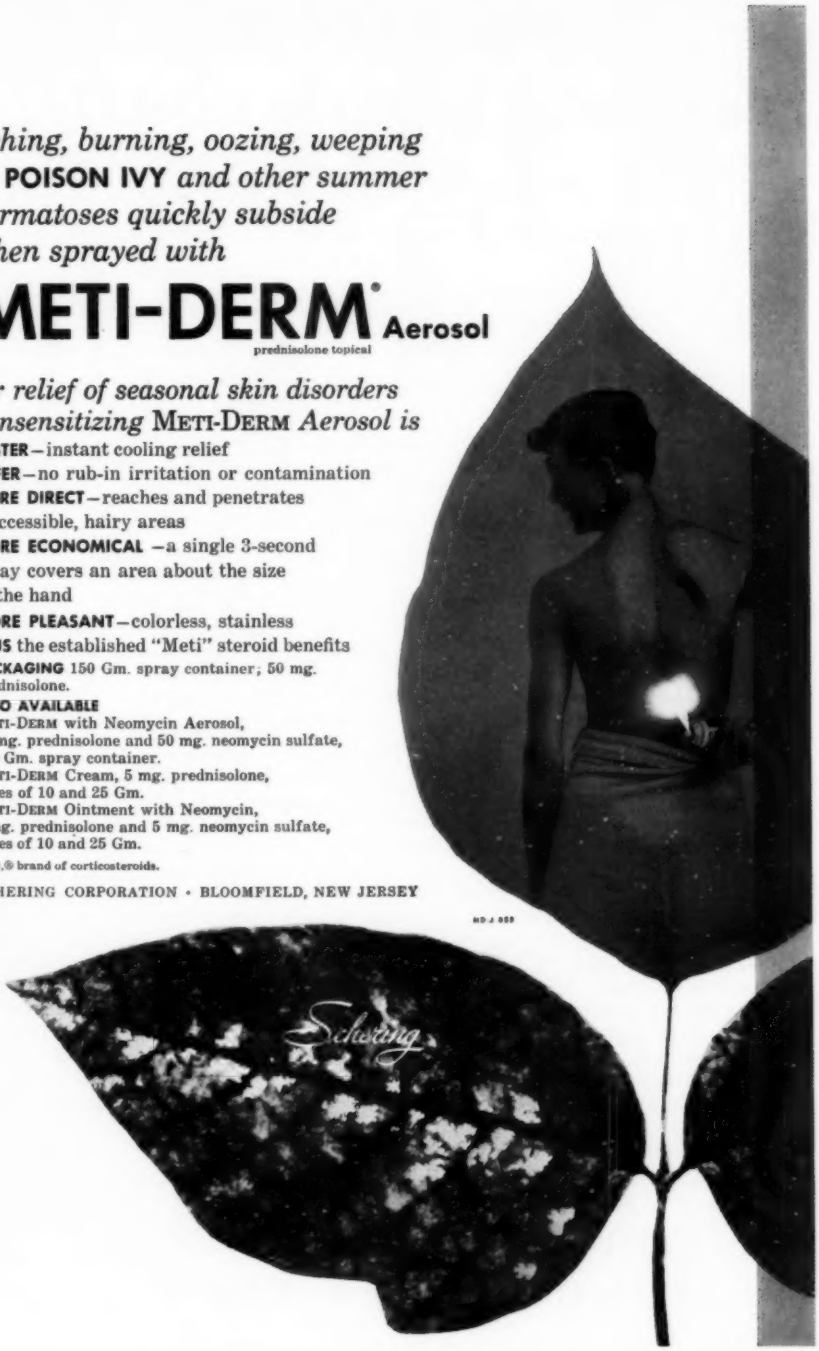

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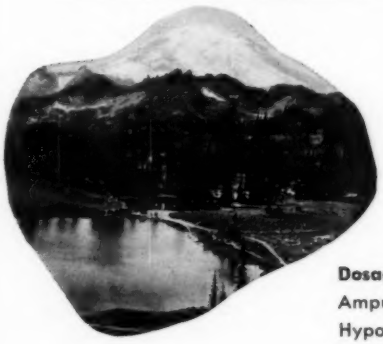

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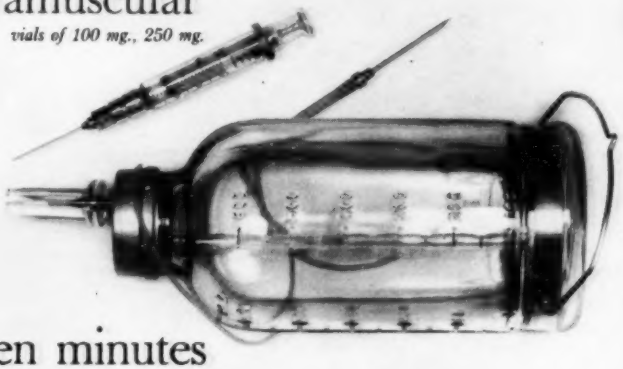

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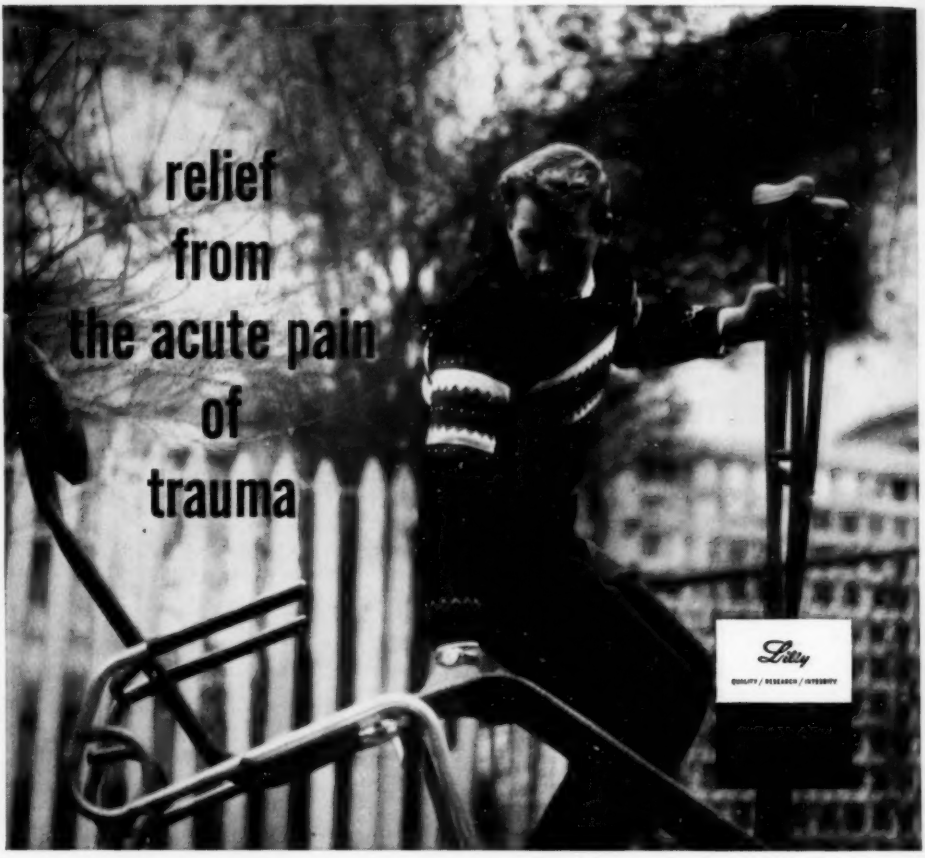

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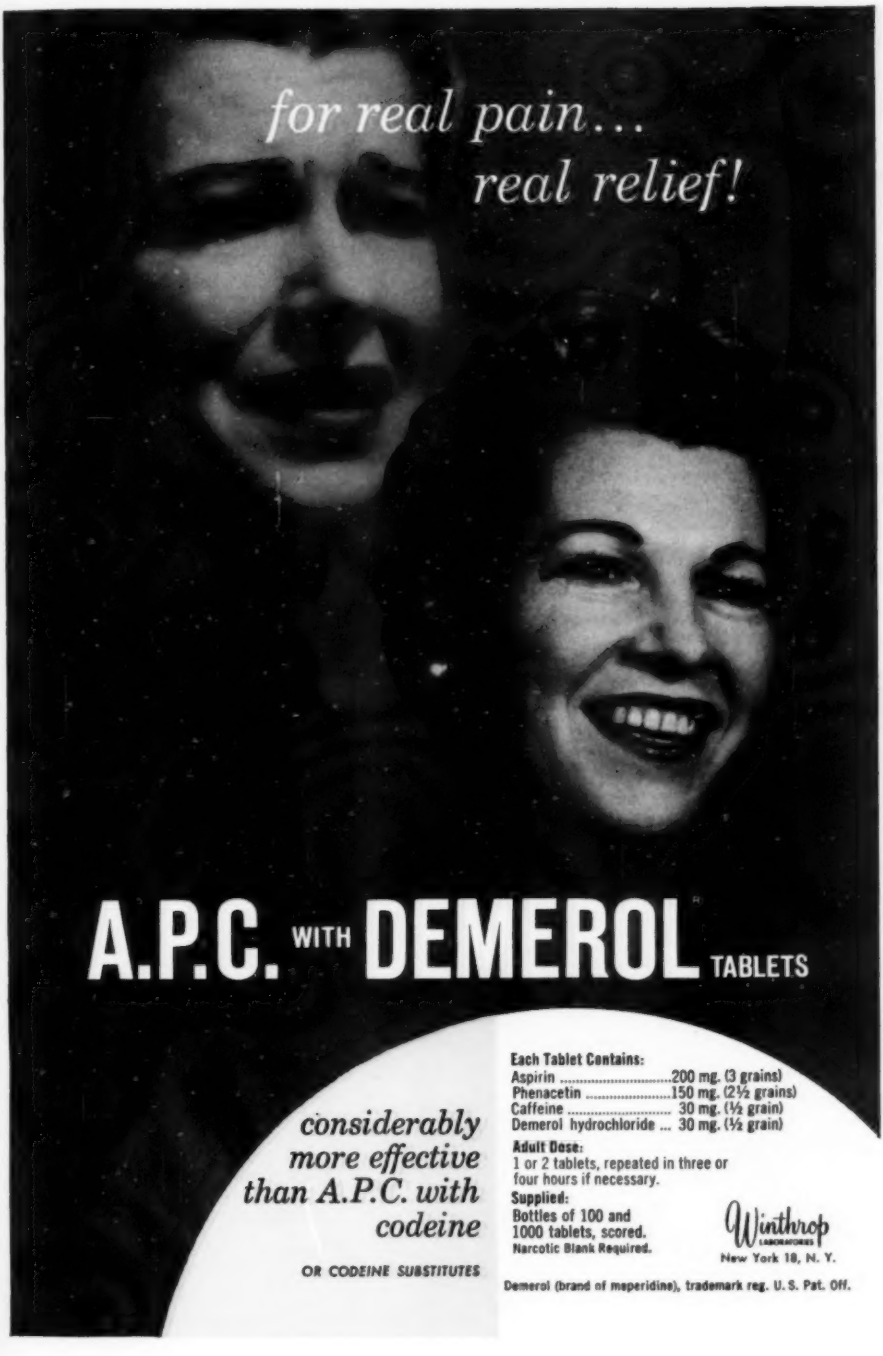
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